Mechanisms underlying visual perception and vision-based decision-making

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A thesis

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...So, then,
in your hands
I deposit
this bundle
of flowers and horseshoes

and say good-bye,

until later on:

or sooner:

until everything
becomes,
becomes song.

Pablo Neruda
From 'Odes about everything'[1]

[1]Neruda, P. 1996. *Fifty Odes.* Austin: Host publications.

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Abstract

The jumping spider *Evarcha culicivora* (Araneae, Salticidae) is unique because, although spiders are incapable of feeding directly on vertebrate blood, *E. culicivora* has a behavioral mechanism enabling it to feed on blood indirectly. *E. culicivora* is a small East African jumping spider that preys on blood-fed female mosquitoes in preference to any other prey, including male mosquitoes and female mosquitoes that have not fed on blood, as well as the far more numerous and similar-looking midges in the vicinity of Lake Victoria. *E. culicivora* distinguishes its preferred prey of blood-fed mosquitoes using visual and olfactory cues, independently of one another. The optical cues by which *E. culicivora* identifies its preferred prey were investigated using lures made from dead prey and by the novel use of 3D animation software to create virtual mosquitoes that were systematically altered. It is apparent that the primary factors influencing the prey-choice decisions of *E. culicivora* include the mosquito's size, its behaviour, the shape of its abdomen, the appearance of its antennae, and possibly the colour of the mosquito's blood-filled abdomen. *E. culicivora's* preference for blood-fed female mosquitoes appears to be specifically due to the blood within the mosquito rather than the prey's size. This is the first report of a spider actively seeking out blood meals by preferentially preying on blood-fed mosquitoes. However, small juveniles of *E. culicivora* have a further dietary specialization: they prefer blood-fed females of the genus *Anopheles*. In the absence of odour cues, juvenile *E. culicivora* distinguish blood-fed *Anopheles* mosquitoes from mosquitoes belonging to other genera, doing this with eyes of a diameter less than 200 microns. The primary optical cue by which juveniles of *E. culicivora* identify *Anopheles* is by the mosquito's characteristic resting posture. This is the first report of any predator seeking out *Anopheles*, the vectors of malaria, as preferred prey. Furthermore, small juveniles of *E. culicivora*, but not large juveniles or adults, have an effective *Anopheles*-specific predatory tactic for capturing *Anopheles* that is not used when capturing mosquitoes belonging to other genera. *Evarcha culicivora* has a further behavioural trait that is unique among salticids; they are attracted to the odour of a particular plant, *Lantana camara*. However, when in the vicinity of *L. camara*, *E. culicivora* exhibits a profound behavioural change in which it becomes indiscriminate in its choice of prey. A proximate cause of the behavioural change was found to be β-caryophyllene, a volatile produced by *L. camara*. This is the first report of a single environmental chemical cue affecting the prey-choice behaviour of a spider.

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CHAPTER ONE

Introduction

Coalescing cognition, mosquito biology, vision-based predatory behaviour and the mind-altering effects of plant-volatiles is the task of this thesis. My study animal, *Evarcha culicivora* (Wesolowska & Jackson, 2003), is an aptly named mosquito-eating jumping spider (Salticidae) native to the shores of Africa's Lake Victoria. No more than about 7 mm long, this spider's predatory behaviour belies a complexity that carries implications from the study of optics through to the biological control of malaria. The prey-choice decisions of *E. culicivora* are the subject of this thesis.

Jumping spiders are a well-studied group of animals. As their name implies, the spiders in this family, Salticidae, jump to escape from their enemies and to pounce on their prey once they have stalked it, solitarily and quietly, leopard-like. However, it is not their jumping ability that commands attention as much as their outstanding vision. To be able to leap and land directly on prey, from some distance, jumping spiders must have good binocular vision. Differentiating the prey that they are attacking from a rival, or a mate, also requires high visual acuity. Furthermore, all this information must be processed by an appropriate cognitive decision-making apparatus. All this occurs in an animal smaller than a fingernail. A logical question arises: how? The work in this thesis is a step toward answering this question.

E. culicivora is from Uganda and Kenya, a region of endemic malaria (Gouagna *et al.*, 2004), and it is a fascinating animal, not only because of its cognitive capacity, but because of its potential relevance to research on the control of malaria. Chapter 2 is an overview of this devastating disease, its impact, its cause and its vector, mosquitoes in the genus *Anopheles*. The remainder of this thesis is divided into two sections, 'Prey preferences of *E. culicivora*' (Chapters 3-7) and 'Plant-odour effects on *E. culicivora's* prey-choice behaviour' (Chapter 8-10).

Anopheles is a common prey of *E. culicivora* in the wild (Wesolowska & Jackson, 2003). In Chapter 3, I consider *E. culicivora's* basic prey-choice behaviour and how these spiders differentiate between mosquitoes and other prey. I consider whether *E. culicivora* differentiates between male and female mosquitoes and between blood-fed female mosquitoes and female mosquitoes that have not recently fed on blood. In the tests in this chapter, both vision-based and odour-based decision-making was investigated.

In Chapter 4, I further clarify the vision-based prey-choice behaviour of *E. culicivora* of different sizes (and ages). By using lures made from dead mosquitoes and virtual lures made using 3D animation software, I tested the optical cues that *E. culicivora* uses to make prey-choice decisions. By systematically altering individual variables, I consider the cues by which these spiders distinguish between different types of prey.

In Chapter 5, I consider the prey-choice behaviour of *E. culicivora* toward *Anopheles* in particular, an issue of potential relevance for efforts to control malaria. I provide evidence that among juveniles of *E. culicivora* there is a specific preference for *Anopheles* over other mosquitoes. By using 3D animation techniques, I test *E. culicivora* with 'virtual prey'. In particular, I investigate precisely which cues are used by juveniles of *E. culicivora* to distinguish anopheline from non-anopheline mosquitoes.

In Chapter 6, I demonstrate that small juveniles of *E. culicivora* use a specific predatory tactic for catching anopheline mosquitoes. This predatory tactic is restricted to use on mosquitoes belonging to the genus *Anopheles* and is not used by larger juveniles or by adults of *E. culicivora*. This is the first example of a prey-specific predatory tactic that is used only by some members (small juveniles) of a species of salticid. In this chapter the predatory behaviour of small juveniles of *E. culicivora* is described and compared with the predatory behaviour of large juveniles and adults of *E. culicivora*.

In Chapter 7, I challenge the orthodox view that it is practically impossible to study colour vision in non-human animals. I argue that animation techniques are well-suited for studying the ability of non-human animals to discriminate between different wavelengths and provide data to support my argument. Virtual prey, made with 3D animation, in which the prey's colour differed, were shown to *E. culicivora* of different sizes (and ages). I argue that *E. culicivora* has the ability to detect long wavelength light but that *E. culicivora's* ability to distinguish colours improves with increasing eye-size. Preliminary measurements of spectral data were made using a manual spectrometer; however, due to an unexpected delay in the receipt of the spectrometry equipment, detailed spectral analyses of the reflected wavelength of the different colours presented to *E. culicivora* were not done.

Many salticids are known to feed on nectar (Jackson *et al.*, 2001) but little is known about how any salticid species responds to plant odours. In Chapter 9, I show that *E. culicivora* is attracted to the odour of the plant *Lantana camara*, a common introduced shrub on which mosquitoes and *E.*

culicivora nectar feed (RRJ, pers. comm.), but is not attracted to the odour of another plant commonly found in *E. culicivora's* habitat, *Striga hermonthica.*

Chapter 10 considers the relationship between plants, mosquitoes and spiders. In particular, I consider the effects of the volatiles of *Lantana camara* on the prey-choice behaviour of *E. culicivora.* Based on *E. culicivora's* preference for the odour of *L. camara* (Chapter 9), and based on *E. culicivora* often being found on this plant in nature, I investigate the effects of *L. camara* on *E. culicivora's* prey-choice behaviour. Vision-based prey-choice tests in the presence and in the absence of these plants reveal that *E. culicivora's* prey-choices are different in the presence of these plants. Furthermore, the results of vision-based prey-choice tests using compounds present in the headspace of *L. camara* suggest that the sesquiterpene β-caryophyllene may be a proximate cause in the change in *E. culicivora's* prey-choice behaviour.

In Chapter 11, the effect of β-caryophyllene on *E. culicivora's* behaviour is further investigated in a visual prey-choice experiment. β-Caryophyllene was purified for experiments with adults of *E. culicivora.* Virtual prey, made using 3D animation software, were used to determine the effect of *L. camara* flowers, β-caryophyllene, and a control with no odour on *E. culicivora's* behaviour. These experiments were designed to be cognitively demanding, the rationale for this being to determine whether *E. culicivora's* attention state changes in the presence of the odour of *L. camara* and β-caryophyllene. It is apparent that, in the presence of *L. camara* flowers and in the presence of β-caryophyllene, *E. culicivora* makes different decisions. These results are discussed in relation to the literature on the effects of plant volatiles, in particular β-caryophyllene, on other arthropods.

The modest task of the concluding chapter is to synthesise the findings presented in this thesis in relation to the literature.

Finally, I have provided two appendices. The first appendix describes the methods used for creating the 3D animated virtual prey used for this research, presented as a pictographic overview of how these 'prey' were created. In the second appendix, I provide data on the size of the anterior-median eyes of *E. culicivora* of different sizes.

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CHAPTER TWO

Mosquitoes and malaria

The Germ

A mighty creature is the germ,
Though smaller than the pachyderm.
His customary dwelling place
Is deep within the human race.
His childish pride he often pleases
By giving people strange diseases.
Do you, my poppet, feel infirm?
You probably contain a germ.

Ogden Nash (1980)

1. Mosquito biology
    Description
    Lifecycle
    Feeding behaviour
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1. Mosquito biology

Description

Mosquitoes are found in nearly every climatic region of the world, from the Arctic to the Tropics, surviving severe winters or dry seasons, depending on the region. Mosquitoes belong to the family Culicidae in the order Diptera ('two-winged' insects). The family Culicidae is further divided into

the three subfamilies Anophelinae, Toxorhynchitinae and Culicinae (Gillet, 1971). The mosquitoes relevant to this thesis are anophelines and culicines. In this chapter, I will review mosquito biology, giving special attention to the life cycle of *Anopheles gambiæ* (subfamily Anophelinae), the principal vector of malaria in Sub-Saharan Africa. Detailed images of *An. gambiæ* are provided in Appendix I.

Adult mosquitoes have a globular head, most of which is taken up by the compound eyes. In general, male and female mosquitoes are similar in appearance. The antennae of both males and females have numerous whorls of hair (fibrillae) emanating from each antennal segment, but the fibrillae on the antennae of females are less numerous and shorter. Males use these fibrillae to hear the wing-beats of other mosquitoes flying in the vicinity and to identify conspecific females by sound (Clements, 1999). The mosquito's mouthparts are a long thin projecting proboscis, which, in the female, are designed for piercing and sucking blood from vertebrate hosts. Needle-like stylets in the proboscis pierce the host's skin. The male, which feeds on nectar and water, has only rudimentary mouthparts (Klowden, 1995).

Anopheline mosquitoes differ from other mosquitoes in several respects; for the material presented this thesis, the most relevant differences are the appearance of the mosquito's head and the mosquito's resting posture. The palps of anopheline mosquitoes are almost as long as the proboscis, giving the front of the mosquito the appearance of a three-pronged fork. The palps of male mosquitoes, being clubbed (Fig. 1), differ in appearance from those of females and from those of other mosquito genera. The palps of culicine mosquitoes differ from those of anophelines by being shorter than the proboscis (Fig. 2a).

Undoubtedly it is their characteristic resting posture that provides the easiest way to identify *Anopheles* mosquitoes. A resting *Anopheles* holds its hind legs raised and its abdomen angled up at about a 45° angle from the surface on which the mosquito is standing. When in this posture, the anopheline's abdomen forms a straight line with the proboscis (http://www.cdc.gov/malaria/biology/mosquito/; accessed 31/08/04). This characteristic posture readily distinguishes anopheline from culicine mosquitoes. The culicine's resting posture is with the abdomen held parallel to the substrate and the head bent toward the substrate (Fig. 2b).

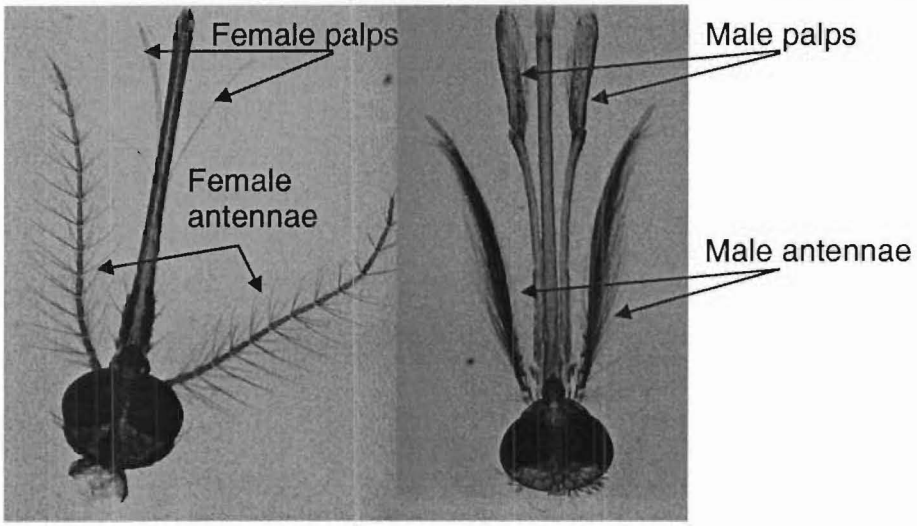

Figure 1. Head of female (left) and male (right) *Anopheles*. Note male's clubbed palps and plumose (feather-like) antennae. From http://www.cvm.okstate.edu/instruction/kocan/vpar5333/533ot5aa.htm (accessed 31/08/04).

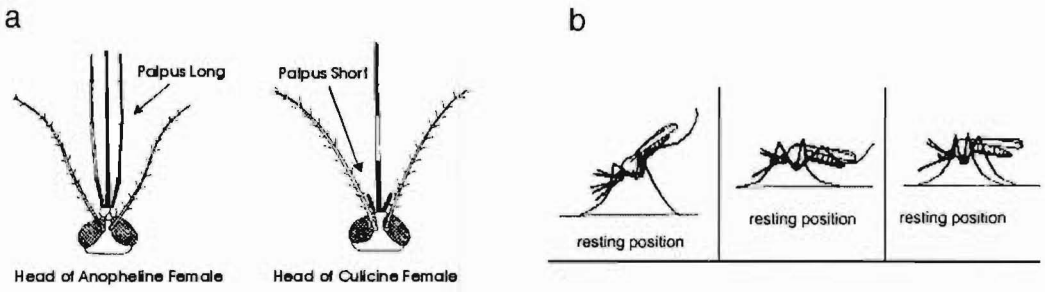

Figure 2. Mosquito characteristics. **a**) Heads of anopheline and of culicine mosquitoes. Note differences in palp length. From http://www.rci.rutgers.edu/~insects/adanat.htm (accessed 01/11/02). **b**) Resting posture of *Anopheles*, *Aedes* and *Culex* (left to right). Note characteristic resting posture of *Anopheles* with the body tilted at an angle to the substrate. From http://www.vnh.org/Malaria/app2.html (accessed 31/08/04).

Life cycle

Mosquitoes undergo complete metamorphosis in which eggs are followed by the larval, the pupal, and finally the adult stage. The length of the complete life cycle is temperature dependent. In warm weather egg-to-adult timing of about 10 days is usual, but several months or longer is typical in species that aestivate or over-winter as larvae (Gillott, 1991; Clements, 1999).

At specific times of the day, typically at twilight, *Anopheles* males form swarms. Swarming males may attract individual females to the swarm by the characteristic pitch of their wingbeats

(Klowden, 1995). *An. gambiæ* males beat their wings at a rate about twice that of conspecific females. Once a female has approached a swarm, a male, detecting the lower wingbeat frequency of the approaching female, probably leaves the swarm and flies towards her, clasping on to her in mid-air (Takken & Knols, 1999). Copulation is brief, usually lasting less than 20 s (Clements, 1999).

Females rely on the blood of vertebrate hosts (especially birds, mammals and reptiles) to stimulate egg production and provide the nutrients required for egg development. Many mosquitoes mate before the female has had her first blood meal, but anopheline mosquitoes usually feed on blood before mating (Takken & Knols, 1999; Charlwood *et al.*; 2003). Females store the sperm in capsule-like spermathecae, releasing a small quantity to fertilize the eggs as they are laid. It is common for females to lay several batches of eggs.

There is considerable variation between mosquito genera in their oviposition behaviour and choice of oviposition sites (Fig. 3). Eggs are usually laid in water. The water might be in a tree-hole, a puddle, inside a discarded tire or even inside an empty snail shell. Eggs hatch after several days to several months (Clements, 1999) and the first instar larva swims free. Anopheline mosquitoes lay boat-shaped eggs singly on the surface of the water (Gillet, 1971) or on moist surfaces (Clements, 1999; Minakawa *et al.*, 2001). Species in the genus *Culex* lay several hundred eggs in rafts (Gillet, 1971; Clements, 1999) that are convex, or boat-like, in shape and are designed to float on the water (Gillet, 1971). Species in the genus *Aedes* lay eggs singly, usually on moist surfaces, although they can withstand severe desiccation (Clements, 1999). The larvae are aquatic and use a pair of spiracles located at the tip of the abdomen for breathing. Mosquito larvae are usually filter feeders, although predacious larvae are also known, especially in the Toxorhynchitinae (Gillet, 1971). Competition for food is reduced by a stratification of feeding levels in the water column (Gillet, 1971). Consequently, in species that feed well below the surface of the water (e.g., *Culex* spp.) the spiracular openings are placed at the tip of a rigid breathing siphon, while surface-film feeders, such as *Anopheles* spp., have spiracular openings directly on the body surface itself (Gillet, 1971). There are four larval instars, after which the mosquito becomes a non-feeding pupa.

The active, comma-shaped pupa has a curved tail that ends in a pair of paddles that it uses for locomotion. Unlike the larval stages, the pupa typically rests at the surface of the water, but it will actively move down in the water when threatened. The pupa breathes through a pair of respiratory trumpets located on its head. During the pupal stage, the mosquito's flight musculature develops and there are numerous other transformations. After a few days the pupal skin splits along the dorsal surface and the adult emerges. Once the cuticle of the newly emerged adult has hardened,

the mosquito flies off and, within a few days after emergence, the adult is ready to mate (Gillet, 1971).

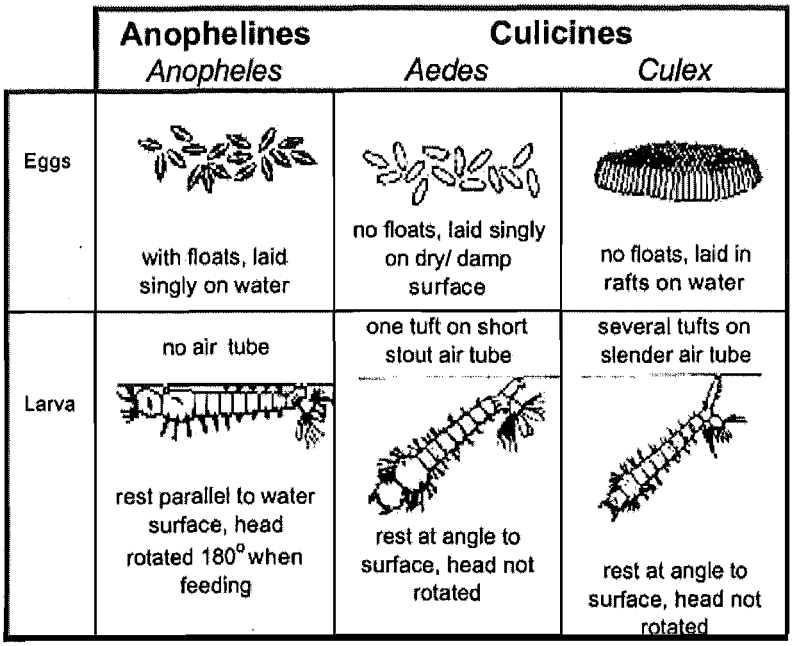

Figure 3. Eggs and larvae of *Anopheles*, *Aedes* and *Culex*. From http://www.vnh.org/Malaria/app2.html (accessed 31/08/04).

Feeding behaviour

Filter-feeding by the larval stage is often inadequate to accumulate sufficient protein to provide a reserve that can persist through metamorphosis into adulthood. Consequently, adult females tend to lack the protein needed to synthesize yolk and develop eggs (Clements, 1999). Feeding on carbohydrate from plant sugar sources, such as overripe fruit and nectar from flowers and extrafloral nectaries (Foster, 1995; Clements, 1999; Gary and Foster, 2001; Impoinvil *et al.*, 2004), provides mosquitoes with enough energy for flight. However, in order to reproduce, most female mosquitoes need to feed on concentrated protein that they get from blood meals (Clements, 1999). Lacking the piercing mouthparts of females, males cannot feed on blood, but survive on carbohydrates found in plants (Klowden, 1995). Female mosquitoes locate blood sources amid considerable background noise in the environment. Female *Anopheles gambiæ* achieve this by using powerful sensory organs on their antennae and on their maxillary palps to detect semiochemicals. A range of host cues is known to attract mosquitoes. Mosquitoes rely on carbon dioxide and other volatile chemical cues from the host for long-range host detection. Having detected these volatile cues, the mosquito initiates an upwind zigzag flight path towards the host. As the mosquito nears the host, other cues, such as visual cues from host appearance, heat, and the water content of the skin may guide

subsequent homing behaviour (Takken & Knols, 1999; Clements, 1999). Carbon dioxide emitted during exhalation by the host is also an important navigation cue when the mosquito is close to the host (Murphy *et al.*, 2001). Anthropophagous species (i.e., species that feed on human blood), such as *An. gambiæ*, are more specifically attracted to human chemical cues of microbial origin, such as foot odour and breath (de Jong & Knols, 1995) and to sweat-borne ammonia (Meijerink *et al.*, 2001). There is evidence that receptors sensitive to human-based odours are downregulated to, or show reduced sensitivity to, olfactory cues a few hours after blood-feeding (Fox *et al.*, 2001; Takken *et al.*, 2001).

Anopheline mosquitoes typically rest during the day and become active in the evening when the female searches for her next blood meal. This crepuscular activity is mirrored in adaptations in the visual system. Compared with diurnal mosquitoes, *Anopheles gambiæ* has eyes well adapted for activity at low light levels (Land *et al.*, 1997; 1999). Once a host is found, the female locates a blood vessel by repeatedly shoving her stylets into the skin until striking a vessel from which to suck blood. Mosquito saliva prevents clotting and keeps the blood flowing from the wound. It achieves this by containing agents that inhibit blood coagulation and normal platelet aggregation (Hurd, 2003). It is the host body's allergic reaction to the proteins in saliva that causes skin irritation and itching (Klowden, 1995). A female mosquito can ingest as much as four times her own bodyweight of blood in a single feeding bout (Holt *et al.*, 2002). After feeding, the female tends to rest nearby. During this time, the blood is digested and the eggs mature (Clements, 1999).

Mosquito saliva is the medium by which parasites and viruses pass to the host, causing diseases. The most noteworthy human diseases for which mosquitoes are the vectors are malaria, yellow fever, dengue and filariasis. Mosquitoes become infected with these disease agents by ingesting blood from an infected person. The disease agent then develops within the mosquito until the insect becomes infective and can transmit the agent to new hosts. Associations between particular parasites, or viruses, mosquitoes and hosts are often highly specific (Gillet, 1971).

Malaria was once thought to be caused by 'bad air' from marshes. The name, 'malaria' is derived from 'mala aria' in Italian, which translates literally as bad air. This disease is now known to be caused by one-celled micro-organisms belonging to the genus *Plasmodium* which are transmitted to the host by a mosquito vector (intermediate host). *Plasmodium* causes malaria in reptiles and birds as well as in humans and other mammals (Spielman & D'Antonio, 2001). Only four species of *Plasmodium* are implicated in human malaria, of which *P. falciparum* is the most virulent. The vector of human malaria is always a species of *Anopheles* (Gillet, 1971).

As long ago as 500 BC, a Brahmin priest named Susruta suggested that mosquitoes spread malaria (Gillet, 1971). However, it was not until the late 19th century that the role of mosquitoes was firmly established. The prevalent view in the 19th century was that bad air emanating from marshes or swamps, such as those near Rome at the time, was the cause of the disease. In 1880, a French military doctor, Laveran (Fig. 4a), established that *Plasmodium* was the causative agent for malaria, which was having a devastating effect on troops working in Algeria. Laveran published these findings (Fig. 4b) and suggested that mosquitoes were the vector, a hypothesis championed by Patrick Manson. Manson had already, by this time, established that mosquitoes are the vectors for filariasis. Ronald Ross (Fig. 4c) is usually given the primary credit for establishing that mosquitoes are the malaria vectors (Gillet, 1971).

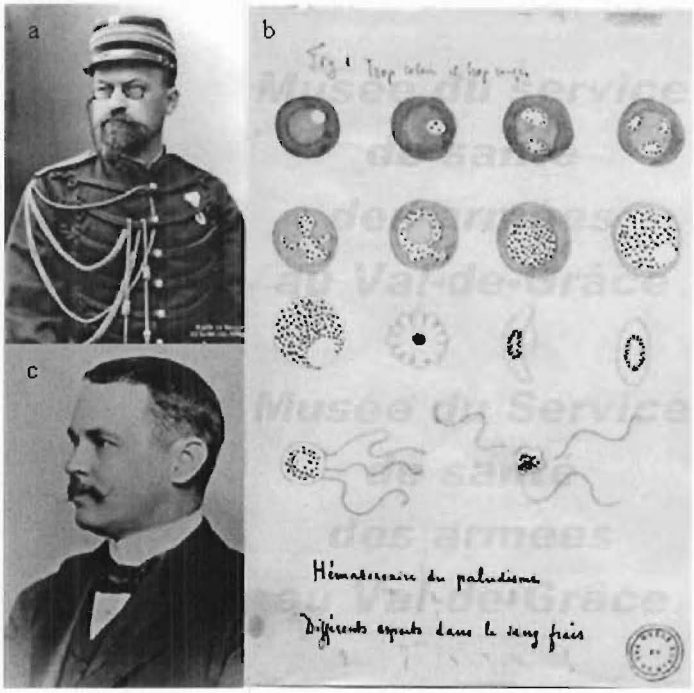

Figure 4. a) Nobel laureate (1907) Alphonse Laveran discovered the causative agent of malaria in 1880. From http://www.cdc.gov/malaria/history/laveran.htm (accessed 01/09/04). **b)** Illustration drawn by Laveran of various stages of malaria parasites. From http://www.cdc.gov/malaria/history/laveran.htm (accessed 01/09/04). **c**) Nobel laureate (1902) Ronald Ross discovered the malarial parasites in mosquitoes. From http://www.nobel.se/medicine/laureates/1902/ross-bio.html (accessed 03/09/04).

Factors favouring parasite transmission

Only a few mosquito species are implicated as vectors of human diseases, of which malaria is the most important. Human malaria is caused by *Plasmodium vivax, P. ovale, P. malariae* and *P. falciparum*, the most virulent species (Phillips, 2001). Of more than four hundred species of *Anopheles*, only about 60 are known to be malaria vectors (Breman, 2001). The most devastating impact of malaria is in sub-Saharan Africa. This is where *P. falciparum*, the parasite that causes the most severe forms of human malaria, is especially common. This is also where *Anopheles gambiæ* is found, and this mosquito is an exceptionally effective vector of *P. falciparum* (Holt *et al.*, 2002).

Human behaviour and resultant environmental modification has played a complex and significant role in spreading malaria and other diseases. The felling of forests has brought mosquitoes into contact with people and travel related to trade has spread infectious agents throughout the world (Gratz, 1999; Gallup & Sachs, 2001; Budiansky, 2002, Sachs & Malaney, 2002). Agricultural practices, such as irrigation and the alteration of waterways, have created novel egg-laying areas for mosquitoes near human dwellings (Gratz, 1999; Breman, 2001). In tropical areas, warm, humid, environments increase the longevity (and hence biting incidence) and breeding capacity of mosquitoes (Breman, 2001; Sachs & Malaney, 2002). Poverty, which is especially typical of tropical areas, leads to poor education about disease transmission and control (Breman, 2001), impedes people from seeking medical aid when needed, and prevents people from adopting what would be simple preventative measures for people in developed countries. Use of bednets is an example. Inexpensive for people from developed countries, without subsidies bednets are rarely used by most people in undeveloped countries (Vogel, 2002a). A higher incidence of malaria, in turn, directly and indirectly leads to lower economic growth (Gallup & Sachs, 2001).

Anopheles gambiæ's biology is closely tied to human dwellings and this has a large impact on this species' capacity as a malaria vector. *An. gambiæ* typically oviposits in temporary sunlit pools, such as the puddles created by human and domestic livestock footprints. These mosquitoes readily enter houses in their pursuit of a blood meal and are adept at getting in through small openings that deter other species (reviewed in Clements, 1999). Females of *An. gambiæ* are highly anthropophagic, endophagic and endophilic. That is, they prefer human blood and they feed and rest indoors. They are highly susceptible to *P. falciparum* infection (reviewed in Takken and Knols, 1999). Coupled with this, they are long-lived, and, unlike most other mosquitoes, they may feed multiple times during a single gonotrophic cycle (Klowden, 1995; Greenwood & Mutabingwa,

2002; Charlwood *et al.*, 2003). *An. gambiæ's* propensity for feeding repeatedly substantially increases its vectorial capacity.

The malaria parasite may modify the physiology and behaviour of the host. These modifications may facilitate the transmission of the parasite to the mosquito. For example, a diseased host's increase in temperature may make the host more detectable by mosquitoes (see Clements, 1999). Parasite manipulation of the mosquito also affects parasite transmission (Morell, 1997; for a review, see Hurd, 2003). For example, *An. gambiæ* infected with *P. falciparum* is significantly more disposed to feed from more than one individual human than uninfected *Anopheles* (Koella *et al.*, 1998).

2. Malaria in Africa

Malaria, the most important insect borne disease in the world (Collins & Paskewitz, 1995), and one of the oldest diseases known to humanity, thrives in impoverished tropical nations around the world (Gallup & Sachs, 2001; Sachs & Malaney, 2002; Miller *et al.*, 2002). The staggering numbers speak for themselves; according to estimates by the World Health Organisation (WHO), over 500 million people are infected with the disease world-wide and 40% of the world's population is at risk of infection (http://www.who.int/tdr/dw/pdf/dw4_2004.pdf, accessed 02/09/04). The death rate in untreated patients is over 10%. More than a million people die of malaria each year (Breman, 2001), over 90% in Sub-Saharan Africa (Greenwood & Mutabingwa, 2002), and 30 times that number need hospitalisation. Despite massive efforts to eradicate malaria in the 1950s and early 1960s, more people are infected with the disease in Africa today than at any other time in history (Trape *et al.*, 2002). Yet, of 272 press releases made by the WHO between January 1999 and 16th July 2002, only five directly concerned malaria (http://www.who.int/inf-pr-2000/en/pr2000-75.html, Press Release WHO/75, 1 December 2000; http://www.who.int/inf-pr-2000/en/pr2000-48.html, Press Release WHO/48, 5 July 2000; http://www.who.int/inf-pr-2000/en/pr2000-46.html, Press Release WHO/46, 30 June 2000; http://www.who.int/inf-pr-2001/en/pr2001-26.html, Press Release WHO/26, 23 May 2001; http://www.who.int/inf/en/pr-2002-31.html, Press Release WHO/31,25 April 2002). Scarce publicity about malaria does not accurately reflect current need for research in the area. After the success of eradicating malaria from most of the Western world in the middle part of the 20th century (see below), malaria-control efforts went into a lull. However, in the past decade, with the African economy and public health systems nearing collapse under the burden of malaria (Sachs, 2002), research has been revived, albeit with little success.

These sobering statistics do not take into account other serious direct and indirect consequences of malaria infections (Fig. 5), which include low birth weight, anaemia (Trape *et al.*, 2002) and cognitive impairment (Snow, 2000), which in turn affects performance and attendance rates at school, with cascading effects of the level of education and consequent well-being of affected societies (Sachs & Malaney, 2002).

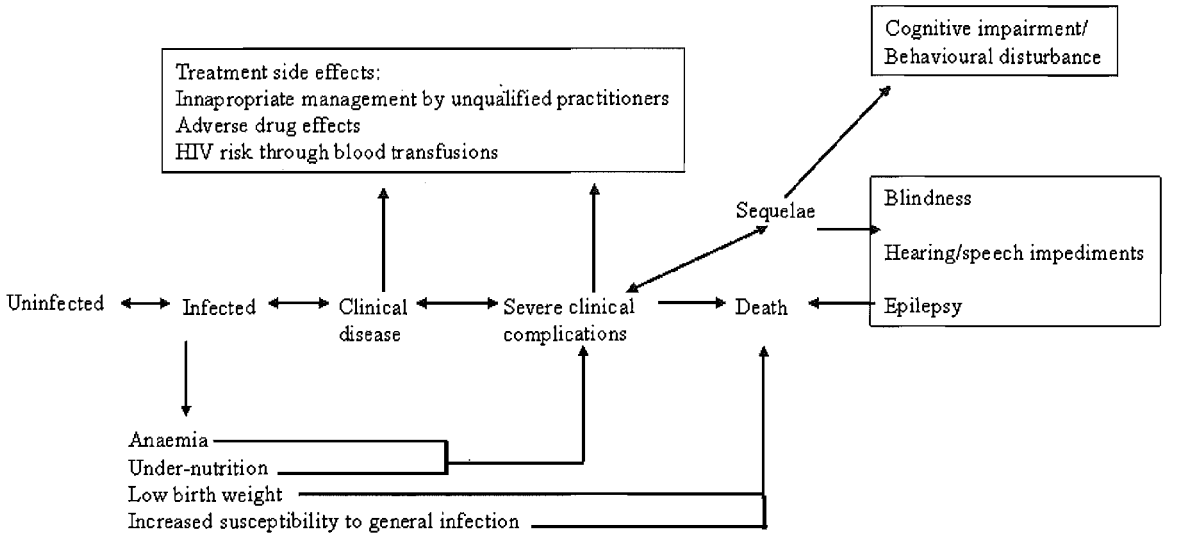

Figure 5. Direct and indirect health consequences of *Plasmodium falciparum* infection. Adapted from Snow, 2000.

3. Malaria parasites; *Plasmodium* life cycle and control

Plasmodium life cycle

Plasmodium falciparum is main cause of disease and death from malaria, followed by *P. vivax* (Mendis *et al.*, 2001). Although there are some minor differences in the infective strategies between *P. falciparum* and *P. vivax*, here I will focus on *P. falciparum*, because this is the principal cause of malaria in Kenya, the field site for the research presented in this thesis.

P. falciparum requires an anopheline mosquito vector to complete its complex life cycle and for its transmission from one human host to another. The life cycle of *P. falciparum* (Fig. 6) is characterised by an asexual cycle in the liver of the human host (liver, or exo-erythrocytic, stage), leading to the release of merozoites into the blood and the subsequent further asexual reproduction that takes place in the red blood cells, or erythrocytes (erythrocytic stage), which is followed by a sexual cycle in the gut of the mosquito host, leading to the formation of infective sporozoites in the salivary glands of the mosquito (Gillet, 1971; Wirth, 2002).

Infection begins with the bite of an infected female *Anopheles* mosquito. Sporozoites released from the salivary glands of the mosquito enter the subcutaneous tissue or directly into the bloodstream during feeding and travel to the liver where they invade liver cells (hepatocytes). During the next two weeks the liver-stage parasites differentiate and reproduce asexually, after which tens of thousands of merozoites burst from the hepatoctye, enter the bloodstream and invade erythrocytes. Individual merozoites that have invaded erythrocytes undergo an additional round of multiplication, producing around 20 merozoites in each erythrocyte. Each one of the new merozoites can invade other erythrocytes. If taken up by the mosquito, merozoites are merely digested by the mosquito (Gillet, 1971).

Symptoms of the disease begin only once the asexual parasite multiplies in the erythrocytes (Sachs, 2002). The clinical manifestations of malaria, fever and chills, are associated with the synchronous (usually late morning) rupture of the infected erythrocytes (Gillet, 1971). The length of this erythrocytic cycle of the parasite life cycle depends on the parasite species: 48 hours for *P. falciparum*, *P. vivax*, and *P. ovale* and 72 hours for *P. malariae*.

A considerable time after the release of merozoites from the liver (Miller *et al.*, 2002), a small proportion of asexual merozoites develop into male and female gametocytes (pre-sexual forms) that are essential for transmitting the infection to others through anopheline (but not other) mosquitoes, but cause no further disease in the human host (Gillet, 1971). Disease gametocytes in the bloodstream are taken up by a female *Anopheles* mosquito during a blood meal. Within the mosquito midgut, the male gametocyte undergoes a rapid nuclear division, producing flagellated microgametes, one of which fertilizes a female macrogamete. The resulting ookinete penetrates the mosquito's gut wall and encysts on the exterior of the gut wall as an oocyst. Soon the oocyst bursts, releasing thousands of daughter parasites, or sporozoites, into the mosquito body cavity which then actively migrate to the mosquito's salivary glands within the thorax, where they remain until being introduced into a suitable human host (Gillet, 1971, Simonetti, 1996).

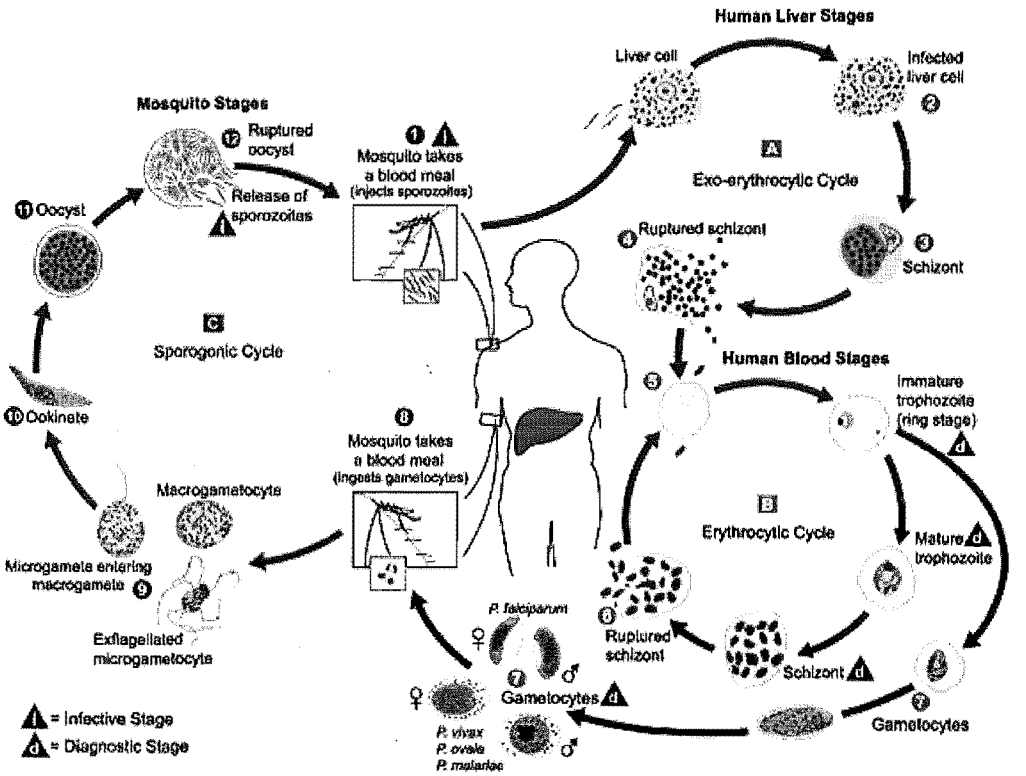

Figure 6. Life-cycle of *Plasmodium*, the protozoan that causes malaria in humans. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶. Sporozoites infect liver cells ❷ maturing into schizonts ❸, which rupture and release merozoites ❹. After this initial replication in the liver (exo-erythrocytic schizogony Ⓐ), parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony Ⓑ). Merozoites infect red blood cells ❺. Ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle Ⓒ. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾ which become motile and elongated (ookinetes) ❿. Ookinetes invade the midgut wall of the mosquito and develop into oocysts ⓫. Oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle ❶. From http://www.cdc.gov/malaria/biology/life_cycle.htm (accessed 02/09/04).

Control

Predictions derived from mathematical models of vector-borne diseases are not always intuitive (see Ewald, 2000; Read & Taylor, 2001). Virulence is highly dependent on host mobility. If a parasite requires direct transmission from one host to another and the diseased host is severely restricted by illness, the infectious agent will not spread and will be eliminated from the population. In such circumstances, natural selection favours less virulent pathogens because they have better prospects for transmission from host to host. However, if the mode of transmission allows pathogens to reach susceptible hosts even when the diseased host is immobilized by illness, as is the case with vector-borne diseases, natural selection permits higher levels of virulence to succeed, with the proviso that the host must remain alive long enough for a vector to receive the pathogen. This simple argument explains much of the current understanding of disease ecology. This argument explains why, for example, the common cold is not particularly virulent. If a cold had us dying in our rooms we would not be able to cough in public areas. It also explains why vector-borne diseases such as yellow fever, sleeping sickness and malaria are extremely incapacitating.

For a vector-borne pathogen the feeding behaviour of the vector is a critical factor for understanding disease transmission (Simonetti, 1996). Although it may seem counter-intuitive, the population density of vectors is not so especially important for disease transmission but the life span of the vector is very important. In the case of malaria, a mosquito that bites a *Plasmodium*-infected human must live not only long enough to find and bite another person but it must also live long enough for the parasite to undergo its cycle of sexual reproduction in the mosquito midgut for successful re-infection via the mosquito's saliva. In *Plasmodium*, this cycle takes 9-18 days (http://www.cdc.gov/malaria/biology/index.htm, accessed 02/09.04). *Anopheles gambiæ* is an especially effective vector for *Plasmodium falciparum.* Being highly anthropophilic and taking frequent blood-meals, the feeding behaviour of *An. gambiæ* ensures that parasites will encounter their human host frequently. It is also a mosquito with an exceptionally long life span (Gary & Foster, 2001; 2004). This combination of longevity and a specific feeding preference makes *An. gambiæ* the world's most efficient malaria vector (Miller & Greenwood, 2002).

Epidemiological models often assume that the parasite is benign to the vector (e.g., that *Plasmodium* does not harm *Anopheles*), based on the idea that parasite fitness is linked to vector fitness (Hurd, 2003). However, this is a controversial argument (see Ferguson & Read, 2002a,b). Some studies on malaria vector-parasite interactions suggest that *Plasmodium* reduces the vector's fecundity. Competition for nutrients, for example, might have this effect.

There have also been controversial arguments about parasite manipulation of *Anopheles* (i.e., instances of *Plasmodium* influencing *Anopheles* in ways that increase chances of the parasite undergoing the full developmental cycle and being transmitted into the next host). For example, *Plasmodium* might increase *Anopheles'* longevity (see Hurd, 2003).

Both *Anopheles gambiæ* and *Plasmodium falciparum* most likely originated in Africa and coevolved with humans (Conway *et al.*, 2000; Gallup & Sachs, 2001). Although numerous other species of both the parasite and its vector are found in Africa (that obviously exacerbate the malarial burden), the fact that the most efficient vector for the parasite (*An. gambiæ*) and the most virulent parasite species (*P. falciparum*) are prevalent in Africa ensures that malaria will be an ongoing problem.

If, on average, each infected human transmits malaria to fewer than one other person, the *Plasmodium* population will not be able to sustain itself and the epidemic will logically be broken. In the US, massive mosquito control programmes based on DDT spraying, coupled with the installation of window screens, achieved this goal in 1954 (Ewald, 2000; Budiansky, 2002), only five years after the US Congress allocated the 7 million dollars for a DDT-eradication programme (Garrett, 1994). Malaria has also been effectively eliminated from other regions by similar methods, including in Europe, Australia, and most of the temperate areas of Asia. Cold winters facilitated elimination of malaria from temperate zones because the period required for the mosquito-host stages of parasite development increases as the ambient temperature declines. Generally, for human malaria, the parasite requires the temperature to be above about 18°C for development and *P. falciparum* requires even higher temperatures, above about 20°C (Sachs & Malaney, 2002; Miller & Greenwood, 2002). Winters in temperate regions prevent year-round prevalence of *Plasmodium*, but it is common in the Tropics to find regions where the mean minimum temperature is above 18°C, even 20°C, throughout the entire year. In sub-Saharan Africa vast areas have a temperature regime that is optimal for *P. falciparum* (Fig. 7). The availability of water for breeding by mosquitoes appears to be less important than temperature because *Anopheles* lays eggs in either temporary or permanent bodies of water (Vogel, 2002b), as well as in moist soil (Minakawa *et al.*, 2001), ensuring that people experience perennial exposure to mosquitoes (Fig. 7). In some areas, such as the Lake Victoria region of Western Kenya, the study-site for the work presented in this thesis, the entire (100%) population is infected (Fig. 8).

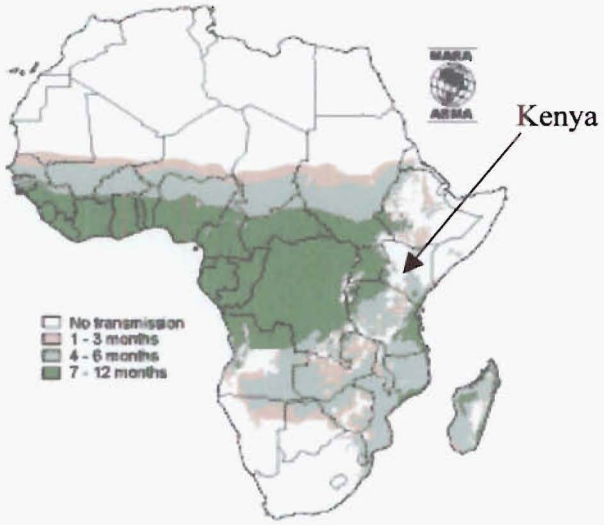

Figure 7. Duration of malaria transmission seasons across Africa. SW Kenya in dark green region (7-12 month transmission). From http://www.rbm.who.int/cmc_upload/0/000/015/370/RBMInfosheet_3.htm (accessed 02/09/04).

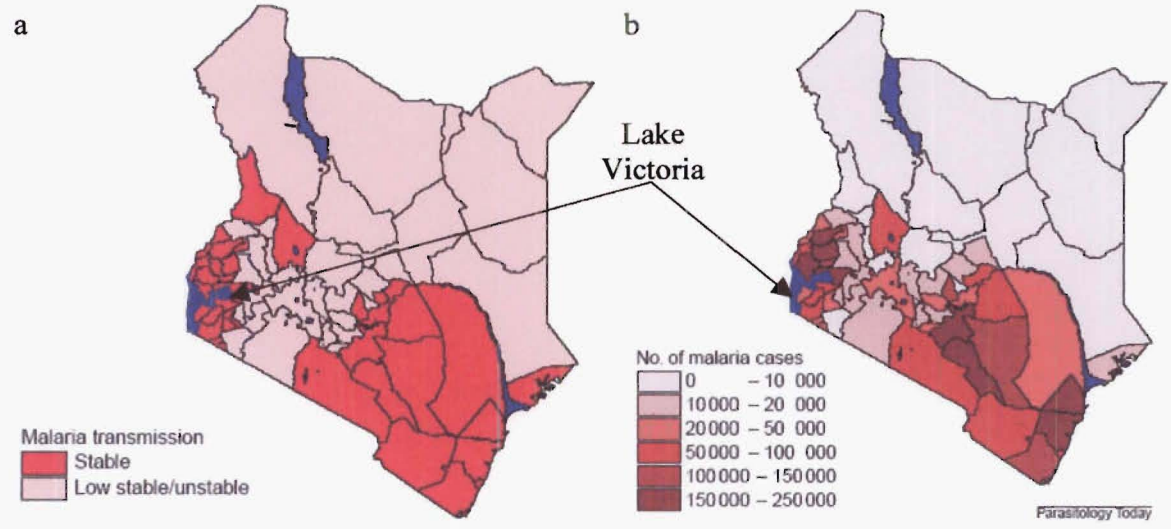

Figure 8. Map of Kenya. Lake Victoria in left-hand corner (SW Kenya). **a)** Estimates of low, unstable malaria (pink) and high-medium stable malaria transmission (red) in Kenya (by district). **b)** Estimated number of malaria attacks experienced by schoolchildren aged 5-14 years. Adapted from Brooker *et al.* 2000.

Other factors conspire against the eradication, or even the control, of malaria in tropical and subtropical regions, with Africa being particularly problematical. The initial success of DDT spraying and the profligate use of chloroquine-based treatments for malaria in the 1950s and 60s left

people with an undue sense of optimism. It must have been a truly inspiring era: diseases such as polio, smallpox and malaria were being eradicated in many areas of the world, the Green Revolution was underway to provide people in third-world countries with enough rice, and other cash crops, to sustain their populations, and humans landed on the moon. By the early 1960s, it was clear that things would not work out as planned. Drug-resistant mosquitoes and pathogens had emerged and the rice paddies of the Green Revolution became perfect oviposition sites for mosquitoes (Garrett, 1994). Forty years later, the situation remains the same, except that now we have practically exhausted the array of chemicals, both for control and for treatment, we can throw either at *Anopheles* or at *Plasmodium* (Trape, 2001; Greenwood & Mutabingwa, 2002). Roll Back Malaria (RBM) is an initiative launched in 1998 by a consortium of the World Health Organisation (WHO), the World Bank, the United Nations Development Program (UNDP) and the United Nations Children's Fund (UNICEF), but it is continually under-funded. The goals of RBM were to halve the mortality toll of malaria by 2010, and further halve it by 2015. These goals are now widely regarded as unrealistic (Sachs, 2002; Sachs & Malaney, 2002). However, the imminent collapse of the first concerted effort to control malaria in four decades (Narasimhan & Attaran, 2003) is inappropriately viewed as an excuse to give up. A major problem from the beginning was under-funding. RMB was based on the expectation of funding by more than USD 2 billion (Sachs, 2002; Narasimhan & Attaran, 2003), but it began with about USD 100 million, a sum that has barely changed since the start of RBM (Sachs, 2002; Narasimhan & Attaran, 2003).

Malaria affects those who can least afford to pay for its prevention or treatment. With little prospect of an adequate commercial return in those parts of the world where malaria prevails, the traditional methods of obtaining drugs through the pharmaceutical industry are simply not realistic. Even if drugs at affordable prices were available in areas of high malaria transmission, drugs seem to be only a temporary solution, at best, as drug-resistant malaria is continually evolving (Trape, 2001; Greenwood & Mutabingwa, 2002).

Vector control measures have been directed against both larval and adult stages of *Anopheles*. Physical measures against larvae include draining standing water, ensuring that drainage channels run freely, sealing drains and removing receptacles containing water such as old tins. Larvicides applied regularly to water surfaces are effective at killing the larval stages before they pupate. Chemical larvicides include petroleum oils, DDT, and organophosphate insecticides, such as temephos (Walker, 2002). Larval biocontrol methods primarily revolve around larvivorous fish, particularly from the family Cyprinodontidae (see Walker, 2002), and pathogens such as *Bacillus*

sphaericus and *B. thuringiensis israelensis* (Moser *et al.*, 2002; Walker, 2002), although novel techniques using essential oils from plants show some promising insectividal activity (Araujo *et al.*, 2003). Because mosquito larvae are relatively non-motile, and adults can avoid treated sites, it has been argued that larval control is the most effective way of combating malaria (Killeen *et al.*, 2002). However, the transient nature of many of the bodies of water that *An. gambiæ* use as oviposition sites (Gimnig *et al.*, 2001) presents a formidable (Walker, 2002), though presumably not insurmountable, challenge.

However, other authors have suggested that the best form of control for malaria is to kill the adult mosquitoes (Curtis & Townson, 1998). Spraying bedrooms with residual-activity insecticides, such as DDT, organophosphates, carbamates and pyrethroids (Guyatt *et al.*, 2002; Walker, 2002) or spraying bed nets with pyrethroid-based insecticides (Curtis & Townson, 1998; Guyatt *et al.*, 2002; but see Trape, *et al.* 2002; Vogel, 2002b) have met with a measure of success in controlling adult mosquitoes. Mosquitoes can also be driven away by the use of repellents and kept out with screening on windows. Traditional control against *An. gambiæ* in Kenya has also included the burning of particular types of plants throughout the night (the period when *An. gambiæ* is most active). There appears to be some repellency in this method. For example, the lemon eucalyptus, *Corymbia citroidora*, a plant containing citronella (Seyoum *et al.*, 2002), is widely used as a mosquito repellent in Africa. On the whole, however, burning plant material does not seem a tractable preventative method because there is little in the way of convincing evidence that malaria is actually reduced by this method (Seyoum *et al.* 2002), it is impractical to re-stock the fire continuously throughout the night and the discomfort of having a fire close by all night in the warm tropics makes this an unpopular mosquito-control measure.

Regardless of whether it is more effective to tackle adult or larval mosquitoes, it is widely accepted that the only way to control malaria in Africa is by interrupting the transmission of the *Plasmodium* parasite (Miller & Greenwood, 2002). Clearly, this goal is not being achieved. Efforts to develop a vaccine for malaria and efforts to replace wild *Anopheles* with transgenic mosquitoes have received a lot of publicity but it is widely acknowledged that neither can be expected to have much impact for well over a decade (Coates, 2000; Alphey *et al.*, 2002; Ito *et al.*, 2002; Miller & Greenwood, 2002; Richie & Saul, 2002; Scott *et al.*, 2002). Although vaccination against viral and bacterial diseases has met with considerable success, vaccines against an eukaryotic organism have so far met with dismal failure (Ewald, 2000; Shiff, 2002).

Despite the initial success of drugs such as chloroquine and quinine to treat malaria, drug resistance in the parasite is now widespread (Hastings *et al.*, 2002a,b; Wellems, 2002). More recently, artemisin (or qinghaosu), a plant-derived terpenoid (Cowan, 1999) has come into wide usage, but the expense of this drug limits its usage in Africa and there are arguments for restricting its use to prevent resistance evolving in *Plasmodium*. We now know that insecticides favour the evolution of resistance in insects and antimalarial drugs favour resistance in the parasite, but, with the simultaneous recent publication of the genomes of *Plasmodium falciparum* (Gardener *et al.*, 2002) and its principal vector, *Anopheles gambiæ* (Holt *et al.*, 2002), new avenues are being opened for immunologists trying to develop malarial vaccines and anti-malarial drugs based on knowledge of these genomes (Kissinger *et al.*, 2002). These developments, however, are long-term goals and there is a need to address this devastating disease and also problems associated with possible genetic modification (GM) 'solutions' (see Alphey *et al.*, 2002). In the foreseeable future, drugs continue to be a major tool for the control and treatment of malaria. Nevertheless, there seems to be a real need for field studies of vector ecology (Curtis, 2000; Enserink, 2002; Shiff, 2002) as this may provide avenues for biological control.

Two types of target genes can be used in transgenic mosquitoes: those that render populations vulnerable to subsequent control measures, such as insecticide susceptibility or temperature sensitivity, and those that interrupt disease transmission by replacing vector with non-vector forms (Simonetti, 1996). Aside from the frequently-raised concerns about releasing any GM organism into the environment, there is the distinct possibility that GM organisms will provide selective pressures on host and, more particularly, parasite, leading to the evolution of counter-defences. After all, *Plasmodium* and *Anopheles* have had a long history of successful counter-adaptation to other 'solutions' used by humans. The failure of DDT to eradicate malaria is a primary example. The notion that genetic modification will provide a magic bullet solution to malaria is dubious. There are strong arguments for a more integrative approach, including the development of combinations of vaccines, effective antimalarial drugs and vector control all as part of the package (Miller & Greenwood, 2002; Shiff, 2002). Bednets, insect repellents and drugs as a cure are, of course, widely used and fairly successful. However, biological control efforts may also be an important part of an integrated approach.

Remarkably little work has been done on more traditional modes of biocontrol outside the use of bacteria and larvivorous fish, both of which target mosquito larvae instead of adults. This endeavour has yielded mixed results (see Walker, 2002), largely because of the transient nature of

pools in which mosquitoes often lay their eggs (Gimnig *et al.*, 2001). Another drawback of these efforts using fish and bacteria is that exogenous species may need to be brought into areas in which they are not endemic, which may disrupt the ecological balance of endemic species.

Mosquitoes have natural predators living sympatrically with them in all parts of the world. Spiders are recognised as major generalist predators of insects the world over (Bristow, 1941; Wise, 1993). Spiders have also been shown to be predators of the larvae of *Culex pipiens* (Breene *et al.*, 1988), the vector for filariasis, West Nile fever and other diseases. However, spiders may play an especially important role as control agents for adult mosquitoes. Strickman *et al.* (1997) suggested that *Crossopriza lyoni* (Pholcidae) may be useful as a biocontrol agent for *Aedes aegypti*, the vector for yellow fever and dengue. Despite this potential, however, spiders seem rarely to have been considered as potential agents of biological control of mosquitoes.

In this thesis, I will consider a salticid spider, *Evarcha culicivora*, of East Africa, that appears to have potential as a biocontrol agent for malaria in Western Kenya and Uganda, two of the most seriously affected locations of *Plasmodium falciparum*-derived malaria on earth.

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CHAPTER THREE

The mosquito terminator, a spider that feeds indirectly on vertebrate blood

Abstract

Spiders are unable to feed directly on vertebrate blood but a small East African jumping spider (Salticidae), *Evarcha culicivora*, appears to have a behavioural mechanism enabling it to feed on blood indirectly. These findings suggest that *E. culicivora* preys on blood-fed female mosquitoes in preference to any other prey, including the far more numerous and similar-looking midges in the vicinity of Lake Victoria. In the absence of odour cues, *E. culicivora* distinguished blood-fed mosquitoes from non-mosquito prey, from male mosquitoes and from female mosquitoes that had not fed on blood. Furthermore, in prey-choice tests on the basis of odour cues alone, *E. culicivora* chose the odour from blood-fed female mosquitoes significantly more often than the odour from other prey, including male mosquitoes and female mosquitoes that had not had a blood-meal. Uniquely, these data support the notion that *E. culicivora's* preference for blood-fed female mosquitoes appears to be specifically due to the blood within the mosquito rather than the prey's size. This is the first report of a spider actively seeking out blood-meals by preferentially preying on blood-fed mosquitoes.

Introduction

No spiders are known to feed directly on vertebrate blood, but *Evarcha culicivora*, a jumping spider (Salticidae) from East Africa, preys frequently on mosquitoes in the field (Wesolowska & Jackson, 2003). Here I investigate a hypothesis suggested by the field data, that *E. culicivora* has innate prey-choice behaviour that enables it to feed on blood indirectly. Female mosquitoes, along with sand flies, black flies, tsetse flies, ticks and other well-known haematophagous arthropods, have specialized mouthparts designed for piercing vertebrate skin and ingesting blood. Spider mouthparts are not designed for this direct style of haematophagy. My hypothesis is that *E. culicivora* lets the female mosquito extract the blood from the vertebrate and, by consistently choosing as prey mosquitoes that are carrying blood, gets blood meals without having to attack the vertebrate animal from which the blood originates. For this hypothesis, it is important to distinguish clearly between diet, choice and preference.

It has become habitual in ecology and behavioural ecology to equate a predator's diet, choice and preference (e.g., Manly, 1974; Roa, 1992). There has also been a long tradition of making casual use of terms such as 'prefer' and 'choose', often with an explicit disclaimer of any cognitive implications. As an effective writing ploy, there is nothing particularly objectionable about using cognitively-loaded words in a non-cognitive context, so long as we can reclaim these words when we need them for making distinctions that actually are related to cognition. For example, Lockwood's (1998) view was that "the relative consumptions of different food types" corresponds closely "with our intuitive definition" of preference (p. 476). Perhaps what is 'intuitive' in ecology is different, but our intuition is that an animal's preference is what it would like to eat and that this allows for the possibility of an animal's diet being different from its preferences. The diets of predators (what the predator actually does eat) may often be influenced by things that do not intuitively correspond to the notion of what the animal wants. For example, a predator may fail to eat its preferred prey when the preferred prey has effective anti-predator defences. 'Preference' is an appropriate word for the predator's attitude toward different types of prey and 'choice' is an appropriate word for behaviour and more specifically a type of behaviour that is driven by preference. Diet may suggest hypotheses about preference and these hypotheses may predict the choices a predator will make in experiments, but data on diet alone do not simply reveal a predator's choices and preferences.

Predators that make vision-based decisions may be especially conducive to experiments testing for prey preferences. Most spiders have poorly developed eyesight (Homann, 1971; Land & Nilsson, 2002), but jumping spiders (Salticidae) are a distinctive exception. These spiders have unique, complex eyes (Williams & McIntyre, 1980; Blest & Price 1984; Blest *et al.*, 1990) that support spatial resolution ability (about 0.04°) without parallel in other animals of comparable size. The highest acuity known for insects (0.4°) is found in a large dragonfly, *Sympetrum striolatus* (Labhart & Nilsson, 1995). Human acuity is 0.007° (Kirschfield, 1976), only about five times better than a salticid's.

Within the Salticidae, pronounced examples of specialized preferences have been documented, especially in species that feed on other spiders and in species that feed on ants. Although the diets of these species include other prey, they consistently choose other spiders or ants, respectively, in laboratory experiments and they can discriminate between prey types by sight alone (Li & Jackson, 1996a).

The salticid genus *Evarcha* Simon 1902 is widespread in the Holarctic, Afrotropical and Oriental Regions and includes more than 50 described species. *E. culicivora* is found only in the vicinity of Lake Victoria in Kenya and Uganda, its typical habitat being a tree trunk or the wall of a building. When quiescent, it hides in the grass or in other vegetation close to the ground, but feeding individuals venture into more exposed locations. *E. culicivora* often enters mosquito-infested houses to feed. However, by far the most abundant mosquito-size insects in these habitats are midges (Chironomidae and Chaoboridae) (Beadle, 1981), known locally as 'lake flies'. My hypotheses are that *E. culicivora*'s preferred prey is specifically female mosquitoes that have recently fed on blood and that it discriminates, not only by sight but also by olfaction, between this particular type of prey and the more common arthropods that do not carry blood, including male mosquitoes which never feed on blood, and including female mosquitoes that have not been feeding recently on blood.

Materials and Methods

General

The field site and laboratory were at Mbita Point in western Kenya, at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (ICIPE). All *E. culicivora* and all mosquitoes came from laboratory cultures. Other arthropods that do not feed on blood were collected from the field as needed (Table 1). For *E. culicivora,* standard spider-laboratory procedures were adopted (Jackson & Hallas, 1986). All testing was carried out between 0800 h and 1100 h (laboratory photoperiod 12L:12D, lights on at 0700).

Laboratory-rearing environments for *E. culicivora* were 'enriched' (spacious cages, meshworks of twigs within each cage) in a manner comparable to that described by Carducci and Jakob (2000). Maintenance diet consisted of letting each salticid feed to satiation on blood-fed female mosquitoes (*Anopheles gambiæ*) and lake flies (Chaoboridae & Chironomidae: see Table 1) three times per week (Monday, Wednesday, Friday). All individuals tested were from second or third generations reared under this regime. Each individual used in any one experiment always had a different combination of parents (i.e., belonged to a different sibship).

Procedures for culturing mosquitoes was as described elsewhere (Gouagna *et al.*, 2004), and all cultures were initiated from specimens collected at Mbita Point. Adult mosquitoes were from one of two groups: 'blood-fed' or 'sugar-fed'. Blood-fed and sugar-fed mosquitoes were maintained on a 6% glucose solution (provided on filter-paper wicks), but blood-fed mosquitoes were also provided with human-blood meals three times per week. All blood-fed mosquitoes had been given blood 4-5

h before being asphyxiated for making lures or before being used as odour sources in olfactometer tests.

Tests were carried out using discrete size classes (1.5 mm, 2.5 mm, 3.5 mm, 4.5 mm, 5.5 mm, 6.5 mm and 7.5 mm) of the adults and the juveniles of *E. culicivora*, size class being determined by body length measured accurately to the nearest 0.5 mm (juveniles, 1.5 to 5.5 mm; adult males, 3.5 to 5.5 mm; adult females, 4.5 to 7.5 mm).

Preliminary testing

Although three different testing protocols (alternate-day, simultaneous-presentation and alternative-prey), and the use of both living prey and lures (dead prey mounted in lifelike posture on cork disks), have been adopted when investigating preferences of araneophagic (spider-eating) and myrmecophagic (ant-eating) salticids (Li & Jackson, 1996b), here findings from only simultaneous-presentation testing (each individual salticid given access to two types of prey at the same time) are presented and only from using lures. In earlier studies on araneophagic and myrmecophagic salticids, findings based on all of these testing methods, regardless of whether living prey or lures were used, have been consistent, but it is the findings from simultaneous-presentation testing with lures that are the least variable and the easiest to interpret. However, preliminary live-prey testing using each of the combinations of prey for which lure testing was done was carried out and the findings were all consistent with the findings presented here from lure tests and significant at $P<0.05$ or better.

In previous studies, simultaneous-presentation testing with lures was carried out using a two-arm (Y-shaped) wooden ramp (Li *et al.*, 1997). Each of the two arms (ends of the Y) of the ramp ended at a perpendicular wooden wall, and there was a lure in front of each wall. There was a different type of lure for each fork of the Y. The salticid chose one of the two lures by walking up the stem of the ramp, turning on to the appropriate arm of the Y and stalking until close enough to attack. However, the salticid sometimes left the ramp before making a choice, and these tests had to be aborted. Here, instead of using the Y-shaped ramp, a more efficient experimental design is used. However, in preliminary testing of *E. culicivora* using the Y-shaped ramp, findings were consistent with the findings I present here.

Vision-based prey choice

Each lure was made by asphyxiating an arthropod with CO_2 and then placing it in 80% ethanol. One day later, the arthropod was mounted in a life-like posture on the centre of one side of a disc-shaped piece of cork (diameter *c.* 1.25 X the body length of the arthropod; thickness *c.* 2 mm). For preservation, the lure and the cork were next sprayed a transparent aerosol plastic adhesive. No individual of *E. culicivora* and no individual lure was used more than once.

The testing apparatus (Fig. 1) was a square transparent glass box (100 mm X 100 mm, X 35 mm high) with a removable glass lid (100 mm X 100 mm). The box had five holes (diameter 16 mm), one centred on each of the four sides and one centred on the box's lid.

A glass vial (50 mm long, diameter 15 mm) was fitted into the hole on each side of the box. The open end of the vial was flush with the inner wall of the box. The other end extended away from the outer side of the wall. There was a lure on each side of each vial (i.e., a total of 8 lures surrounded the box). Each lure faced directly toward the side of the box.

The box was mounted on a wooden platform (170 mm X 170 mm) surrounded by a 40 mm high wooden fence. The fence served as a background against which *E. culicivora* saw the lures.

The entire apparatus was lit by a 200 W incandescent lamp, positioned *c.* 400 mm overhead. Fluorescent ceiling lamps provided ambient lighting. Before testing, the test spider had access *ad libitum* to lake flies and to blood-fed females of *An. gambiæ*. A short pre-test fast (7 days) was adopted, as in the earlier study (Li *et al.*, 1997), the rationale for this being to ensure that the test spiders were motivated to feed during testing.

There were two types of lures present during each test. One type was placed on two opposing sides ('A' positions) and the other type was placed on the adjacent sides ('B' positions). Which of the two lures was placed in positions A was decided at random.

During the majority of tests, the body lengths of all mosquito lures were matched to the nearest 0.5 mm. The objective of these tests was to eliminate prey-size preference as a potentially confounding variable and consider whether *E. culicivora* made prey-choice decisions by which it obtained blood.

In another series of tests, mosquito lures that differed in body length were used. First, the prey-size preferences of *E. culicivora* were determined when the presence or absence of blood in the prey was held constant. Having ascertained prey-size preference, whether preference for blood took precedence over preference for prey size was investigated.

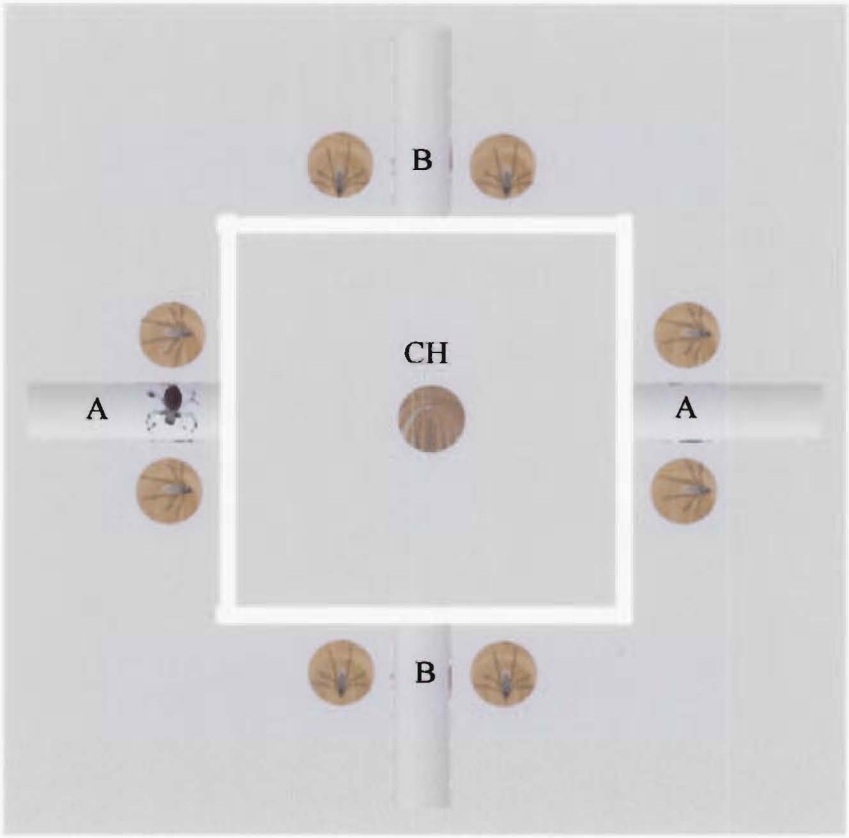

Figure 1. Apparatus used for testing vision-based prey choice. Glass box with central hole (CH), plugged with rubber stopper. Spider introduced through CH. Each of four glass vials (diameter, 50 mm) fits in hole in a side of box. Lures mounted on cork discs and positioned one on each side of each vial. Type of lure at A different from type at B. Criterion for 'choice': spider entered vial and remained there for more than 30 s.

The hole in the lid was used for introducing the test spider into the box. Once the test spider was in the box, the test began and the hole in the lid was plugged with a rubber stopper. Once inside, the test spider was free to move about and enter any of the four vials. Tests lasted 60 min or until the test spider made a choice. When the spider entered, and then stayed inside, any one of the four vials for 30 s, this was recorded as its choice. The rationale for the 30-s criterion was that, in preliminary trials, although individuals of *E. culicivora* often entered a vial for a few seconds and then left, any individuals that stayed in a vial for 30 s remained in this vial for at least 5 min longer and, if they subsequently left this vial, they did not enter and remain in another vial for 30 s or longer.

Between tests, the box and all vials and stoppers were washed with 80% ethanol followed by distilled water, and then allowed to dry.

Olfaction-based prey choice

A Y-shaped olfactometer (Jackson *et al.*, 2002), with airflow adjusted to 1500 ml/min (Matheson FM-1000 flowmeter), was used to assess *E. culicivora's* response to specific odours (Fig. 2). There was no evidence that this airflow setting impaired locomotion or had any other adverse effects on *E. culicivora*'s behaviour. Air was pushed by a pump from a tap through two separate flowmeters into two stimulus chambers and from each stimulus chamber to different choice arms. Air from the two choice arms then converged and moved into the test arm (i.e., the stem of the Y).

Into each stimulus chamber, an odour source (10 prey of the same type) was placed for 30 min before each test. The 30-min pre-test period allowed air to circulate evenly and ensured that air pressure was comparable throughout the olfactometer. A test spider was placed in a holding chamber at the far end of the test arm 2 min before testing began. A removable metal grill fitted into a slit in the chamber roof, blocking access between the test arm and the holding chamber. The grill was lifted to start a test.

Once the grill was removed, the test spider was allowed 30 min to make a choice (definition: entered a choice arm and remained there for 30 s). This 30-s rule has been shown to be reliable in earlier olfactometer studies using salticids (Clark *et al.*, 2000; Jackson *et al.*, 2002), and preliminary testing confirmed that it was reliable for *E. culicivora*.

After each test the olfactometer was dismantled and cleaned with 80% ethanol and then with distilled water. No test spider nor odour source was used more than once.

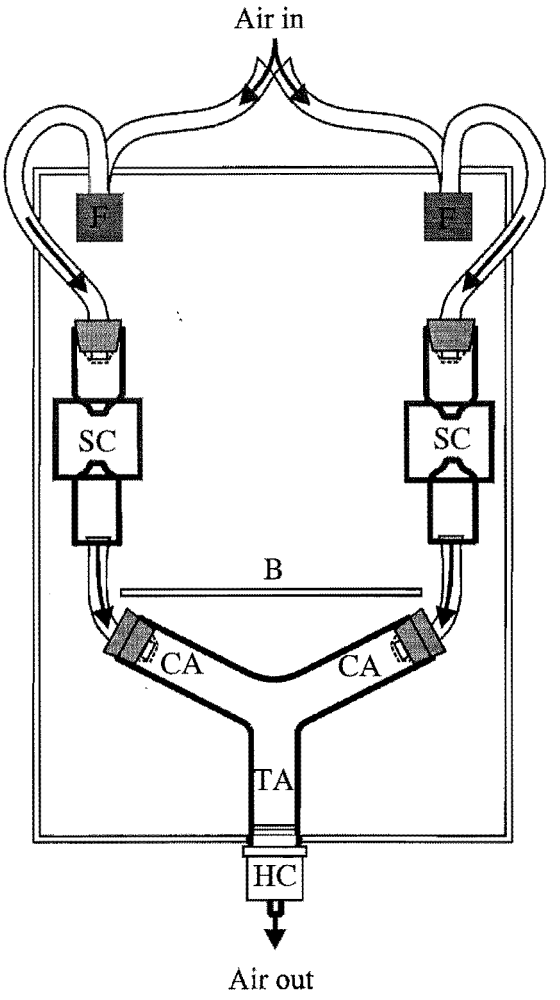

Figure 2. Olfactometer (not drawn to scale). Dimensions of stimulus chambers (SC) (containing odour source): 80 mm long X 70 mm wide X 80 mm high. Holding chamber (HC) (location of test spider at start of test): length 25 mm, internal diameter 25 mm. Start of test: test spider released from HC (grill removed), giving it access to test arm (TA) and to choice arms (CA). Air flows (arrows) from flowmeters (F) through SC and CA. Dimensions of test arm and choice arms: length 90 mm, internal diameter 20 mm. Opaque barriers (B) prevent test spider from seeing odour source. Criterion for 'choice': spider entered CA and remained there for more than 30 s.

On the whole, the arthropods used as odour sources were not especially active during the tests, but the remote possibility that differences in prey activity influenced the choices made by *E. culicivora* cannot be ruled out. However, in another series of tests, prey activity was controlled for by keeping all prey quiescent during the testing period. This was achieved this by immobilizing all prey with CO_2 gas before they were put into the stimulus chamber. If prey began to show signs of activity at any time during the 30-min pre-test period or the 30-min test period, the pump was switched off, the stimulus chamber was disconnected from the rest of the olfactometer and CO_2 gas was introduced into the stimulus chamber, rendering the prey quiescent again, after which the pump was switched on again and the stimulus chamber was connected again to the rest of the olfactometer.

Data analysis

For this study, all data were analyzed using chi-square tests for goodness of fit (null hypothesis: the two choices are made equally often) (Sokal & Rohlf, 1995). Instances in which individuals failed to make a choice in the allotted testing period were rare (never more than 5% for any given prey combination in vision-based or olfaction-based testing).

Results

Vision-based prey choice when body lengths of the two types of prey match

When the alternative was an arthropod other than a mosquito, the juveniles (Fig. 3) and both sexes of the adults (Fig. 4) of *E. culicivora*, regardless of size class (Fig. 5), chose blood-fed female mosquitoes significantly more often than they chose any alternative prey (Table 1). Pooling all data from these tests, 1423 (82.73%) spiders chose the blood-fed mosquito, whereas only 297 (17.27%) chose the alternative prey.

When the alternative was a conspecific male mosquito, *E. culicivora* chose blood-fed female mosquitoes significantly more often than they chose conspecific male mosquitoes. This trend held regardless of the mosquito species and regardless of the sex-age-size category of the individual of *E. culicivora* being tested (Fig. 6). Pooling all data from these tests, 627 (78.38%) spiders chose the blood-fed female mosquito, whereas only 173 (21.62%) chose the male mosquito.

When the alternative was a conspecific female mosquito that had been fed sugar only, *E. culicivora* chose blood-fed female mosquitoes significantly more often than they chose sugar-fed female mosquitoes (Fig. 7). Pooling all data from these tests, 477 (79.5%) spiders chose the blood-fed female mosquito whereas only 123 (20.5%) chose the sugar-fed female mosquito.

Table 1. Arthropods used in laboratory experiments.

Order	Family	Species	Body lengths of individuals used (mm)
Diptera	Culicidae	*Aedes aegypti*	5.0
		Anopheles gambiæ	4.5 & 5.5
		Anopheles funestus	3.5
		Culex quinquefasciatus	4.5
	Chaoboridae	*Chaoborus* sp. (indet.)	4.5
	Chiromonidae	*Ablabesmyia nilotica*	4.5
		Chironomus imicola	4.5
		Clinotanypus claripennis	6.0
		Conochironomus acutistilus	6.0
		Nilodorum brevibucca	4.5
	Tephritidae	*Ceratitis capitata*	4.5
Lepidoptera	Pyralidae	*Chilo partellus*	5.0
Homoptera	Aphididae	*Brevicoryne brassicae*	2.0
Araneae	Oecobiidae	*Oecobius amboseli*	2.0
	Tetragnathidae	*Nephilengys* sp. (indet.)	4.5

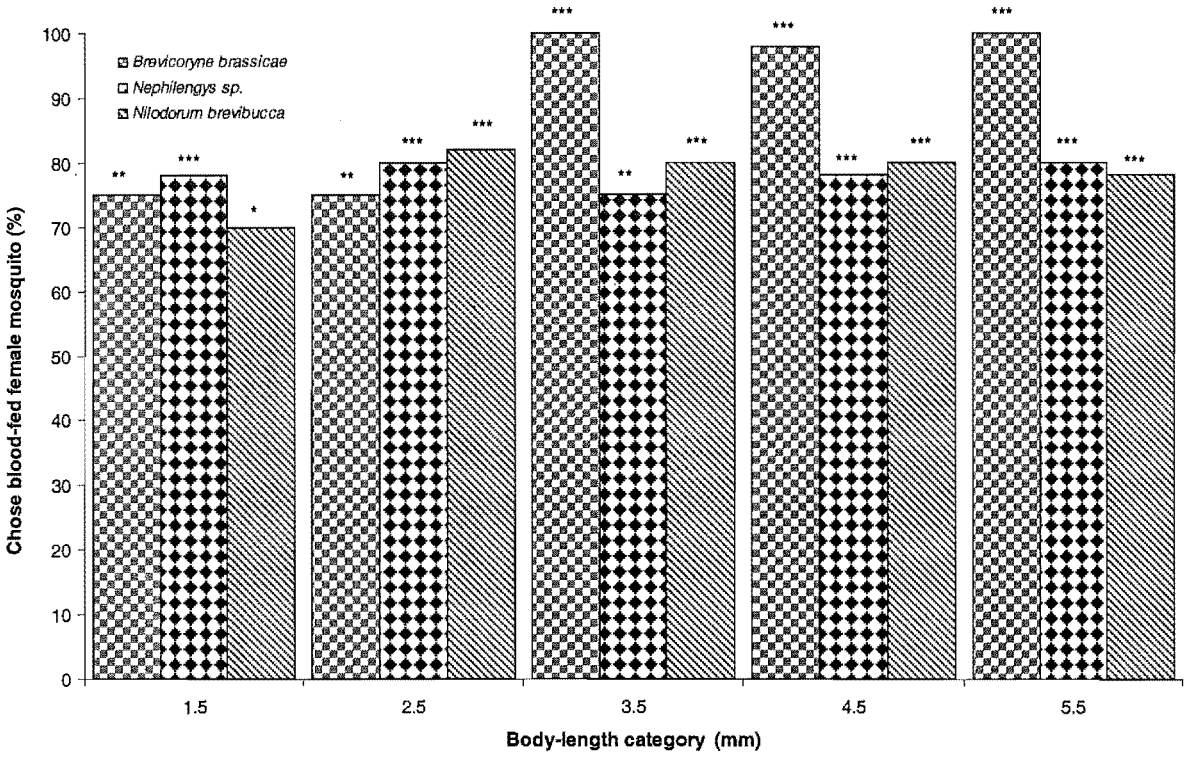

Figure 3. Percentage of times juveniles of different body-length categories of *Evarcha culicivora* chose blood-fed female mosquitoes (*Anopheles gambiæ*, body length 4.5 mm) rather than differently sized categories of other prey. Test spider had simultaneous access to two types of prey in which only visual cues were available. Other prey: *Brevicoryne brassicae* (2.0 mm), *Nephilengys* sp. (4.5 mm), *Nilodorum brevibucca* (4.5 mm). N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01, *P<0.05).

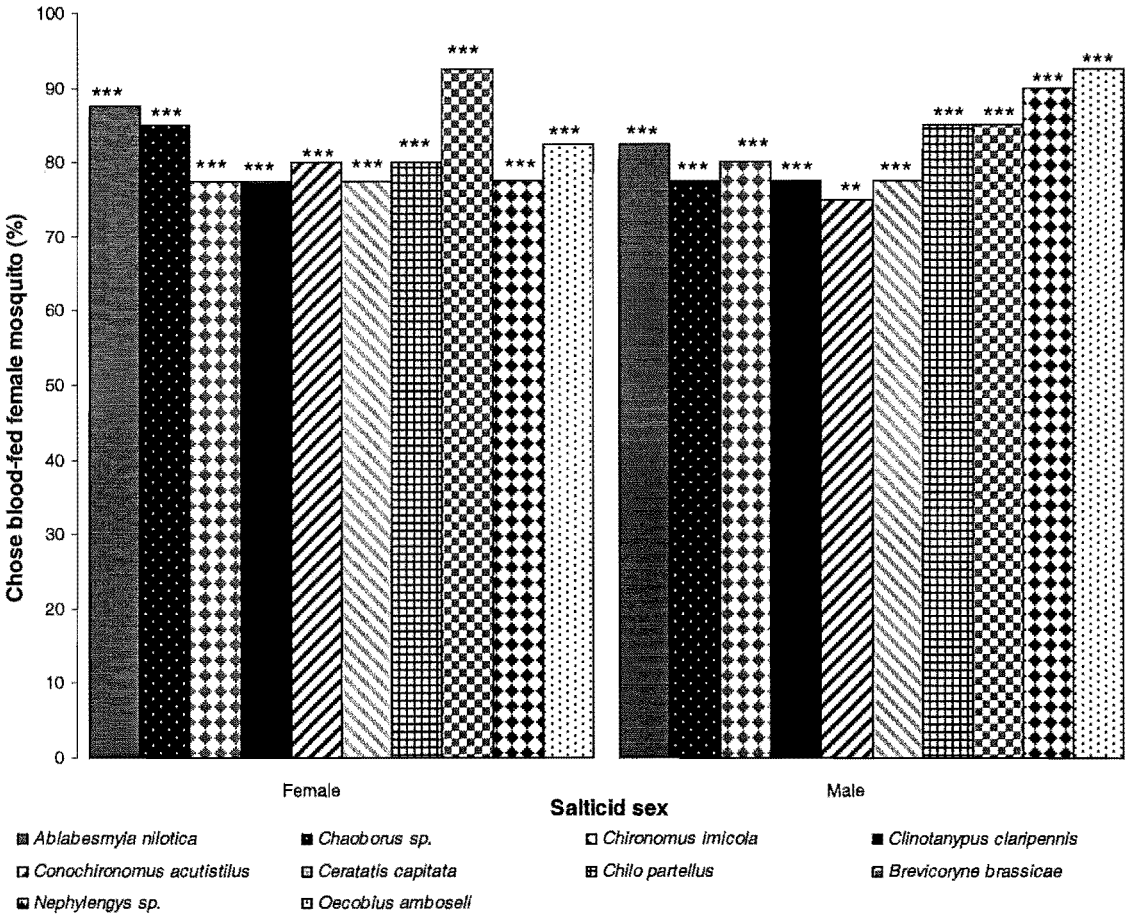

Figure 4. Percentage of times adult males and females of *Evarcha culicivora* (body length 6.5 mm) chose blood-fed female mosquitoes (*Anopheles gambiæ*, body length 4.5 mm) rather than other prey. Test spider had simultaneous access to two types of prey in which only visual cues were available. Other prey as specified by bar striping. Except for *Brevicoryne brassicae*, *Clinotanypus claripennis*, *Conochironomus acutistilus* & *Oecobius amboseli*, body length of other prey matches that of mosquito. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01).

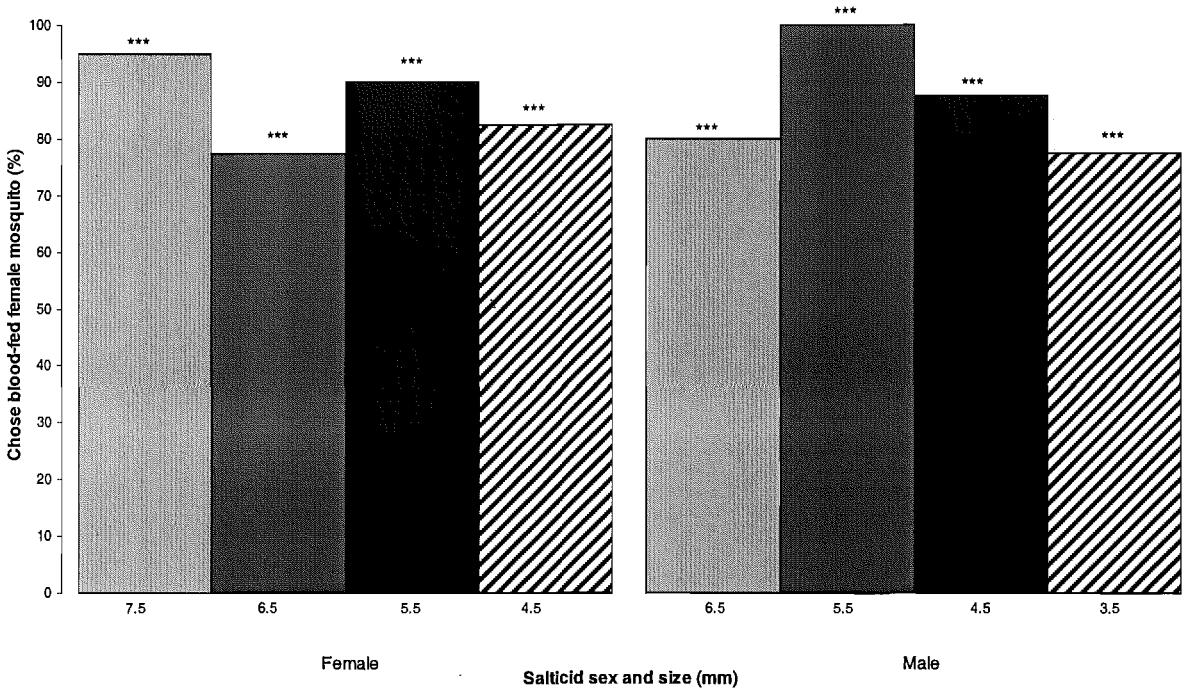

Figure 5. Percentage of times adult males and females of *Evarcha culicivora* (body length variable) chose blood-fed female blood-fed female mosquitoes (*Anopheles gambiæ*, body length 4.5 mm) rather than *Nilodorum brevibucca* (body length 4.5 mm). Test spider had simultaneous access to two types of prey in which only visual cues were available. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001).

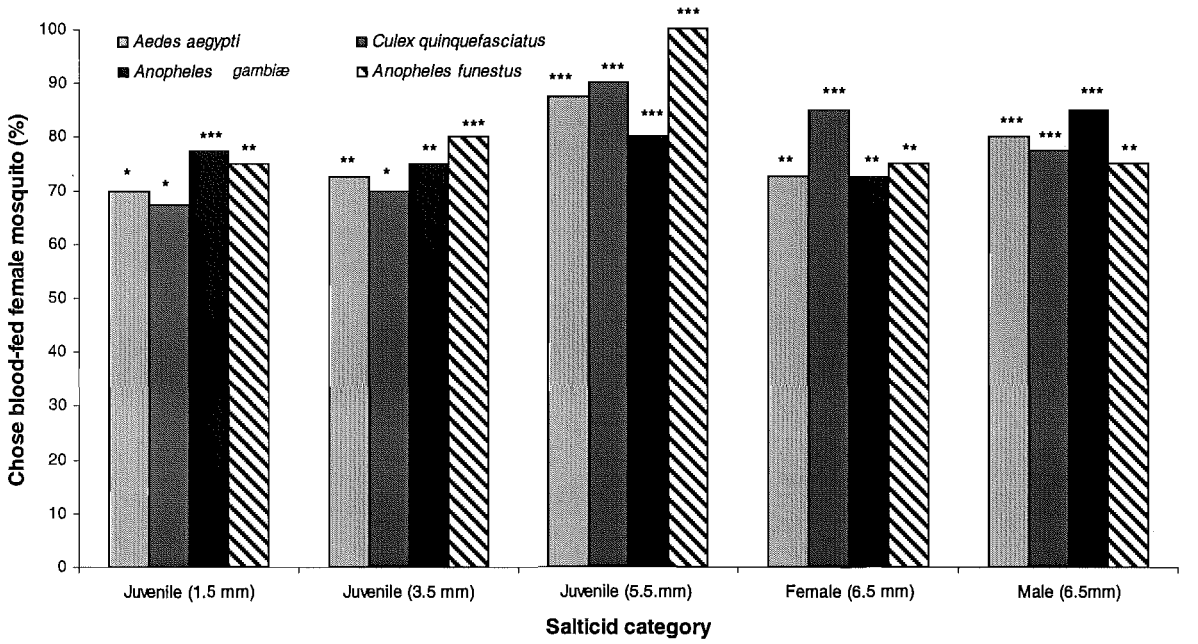

Figure 6. Percentage of times *Evarcha culicivora* (body length and age variable) chose blood-fed female blood-fed female mosquitoes (*Aedes aegypti* 5.0 mm; *Culex quinquefasciatus* 4.5 mm; *Anopheles gambiæ* 4.5 mm; *Anopheles funestus* 3.5 mm) rather than conspecific male mosquitoes. Test spider had simultaneous access to two types of prey matched for size in which only visual cues were available. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01, *P<0.05).

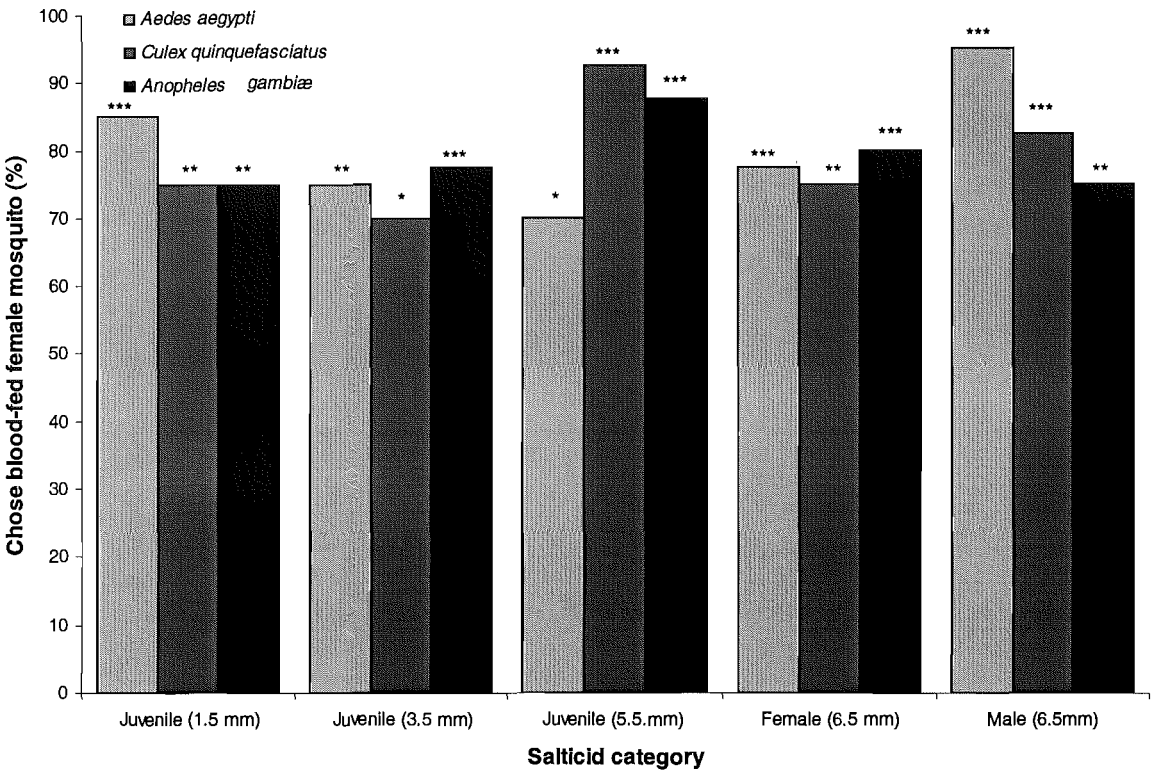

Figure 7. Percentage of times *Evarcha culicivora* (body length and age variable) chose blood-fed female blood-fed female mosquitoes (*Aedes aegypti* 5.0 mm; *Culex quinquefasciatus* 4.5 mm; *Anopheles gambiæ* 4.5 mm) rather than conspecific sugar-fed female mosquitoes. Test spider had simultaneous access to two types of prey matched for size in which only visual cues were available. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01, *P<0.05).

Vision-based prey choice when body lengths of the two types of prey differ

When the alternative was a large blood-fed female mosquito, 1.5 mm juveniles of *E. culicivora* chose the small blood-fed female mosquito. When the alternative was a large sugar-fed female mosquito, 1.5 mm juveniles of *E. culicivora* chose the small sugar-fed female mosquito. The larger juveniles and both sexes of the adults of *E. culicivora*, however, chose the larger blood-fed female mosquito and the larger sugar-fed female mosquito (Fig. 8).

When the large female mosquito was blood fed and the small female mosquito was sugar fed, 1.5 mm juveniles of *E. culicivora* reversed the prey size that they chose. They chose the large, not the small female mosquitoes (Fig. 8).

When the small female mosquito was blood fed, whereas the large female mosquito was sugar fed, larger juveniles and both males and females of the adults of *E. culicivora* reversed the prey size that they chose. They chose the small, not the large female mosquitoes (Fig. 8).

Odour-based prey choice using prey that has not been immobilised

When the alternative was an arthropod other than a mosquito (*An. gambiæ*), the juveniles and both sexes of the adults of *E. culicivora*, regardless of size class, chose the odour of blood-fed female mosquitoes significantly more often than the odour of any alternative prey (Fig. 9). Pooling all data from these tests, 727 (86.55%) spiders chose the blood-fed mosquito odour, whereas only 114 (13.45%) chose the odour from alternative prey.

When the alternative was a conspecific male mosquito, *E. culicivora* chose blood-fed female mosquitoes significantly more often than the male mosquitoes. This trend held regardless of the mosquito species and regardless of the sex-age-size category of the individual of *E. culicivora* being tested (Fig. 10). Pooling data from these tests, 626 (84.5%) spiders chose the odour of blood-fed female mosquitoes, whereas only 124 (15.5%) spiders chose the odour of male mosquitoes. When the alternative was a conspecific female mosquito that had been fed sugar only, *E. culicivora* chose blood-fed female mosquitoes significantly more often than sugar-fed female mosquitoes. This trend held regardless of the mosquito species and regardless of the sex-age-size category of the individual of *E. culicivora* being tested (Fig. 11). Pooling data from all these tests, 252 (90%) spiders chose blood-fed female mosquitoes, whereas only 28 (10%) chose the sugar-fed female mosquitoes.

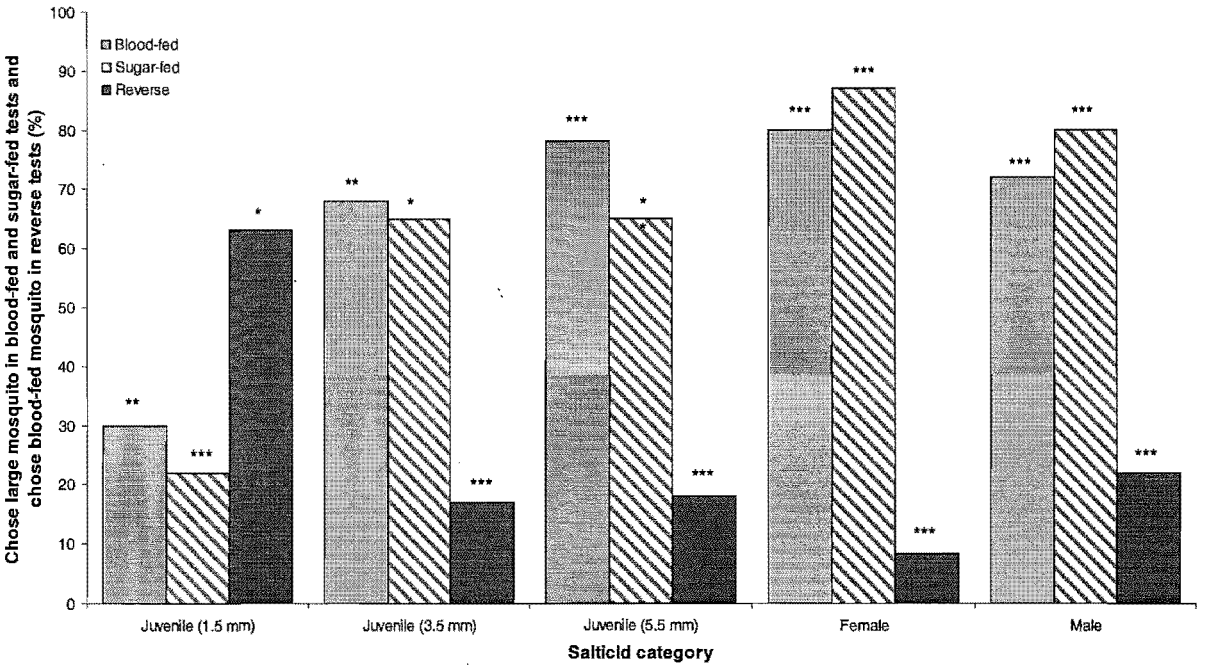

Figure 8. Percentage of times *Evarcha culicivora* (body length and age variable; body legth of adults 6.5 mm) chose blood-fed female blood-fed female mosquitoes rather than conspecific sugar-fed mosquitoes. Test spider had simultaneous access to two mosquitoes of different sizes (*Anopheles gambiæ* 4.5 mm and 5.5 mm). Three tests (blood-fed, sugar-fed, reverse). Blood-fed: large and small blood-fed female mosquito. Sugar-fed: large and small sugar-fed female mosquito. Reverse: 1.5 mm juveniles of *E. culicivora* tested with large blood-fed female mosquito & small sugar-fed female mosquito; adult females, adult males & juveniles larger than 1.5 mm in body length tested with small blood-fed female mosquito and large sugar-fed female mosquito. N=60 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01, *P<0.05).

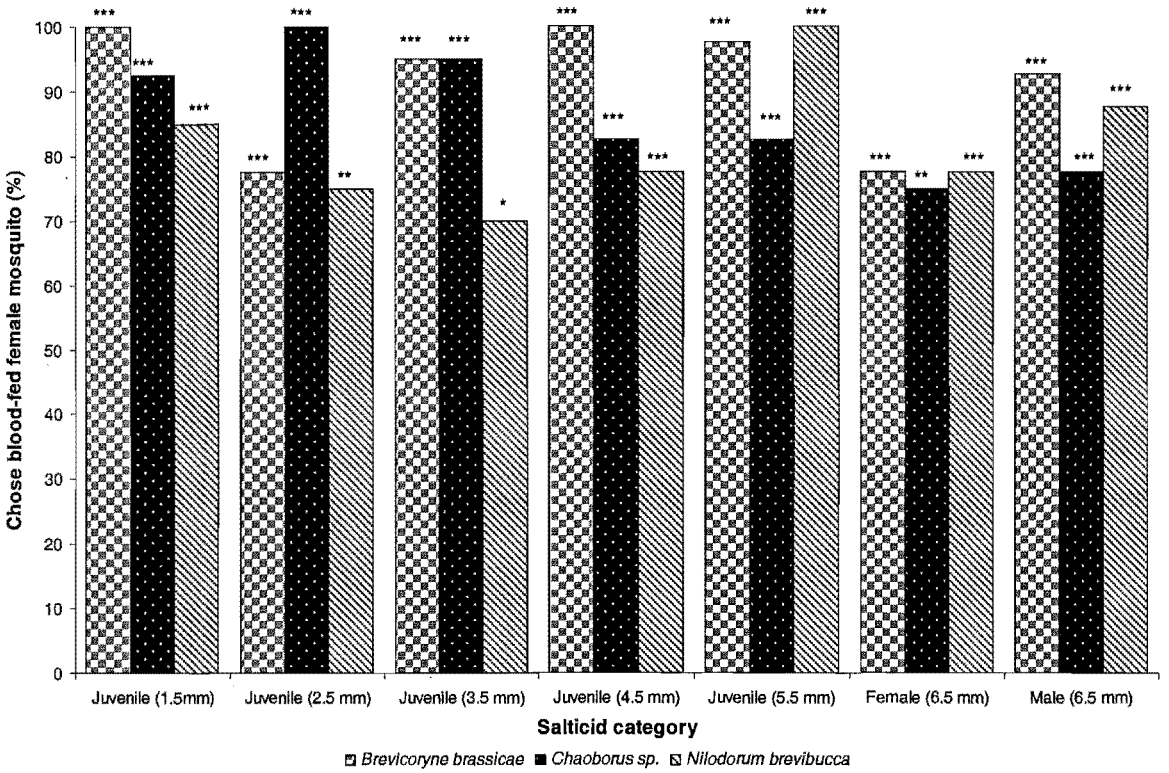

Figure 9. Percentage of times *Evarcha culicivora* (body length and age variable) chose the odour of blood-fed female blood-fed female mosquitoes (*Anopheles gambiæ*) rather than the odour of other prey. Other prey: *Brevicoryne brassicae*, *Chaoborus* sp., *Nilodorum brevibucca*. Test spider had simultaneous access to the odour of two types of prey but no visual cues were available. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01, *P<0.05).

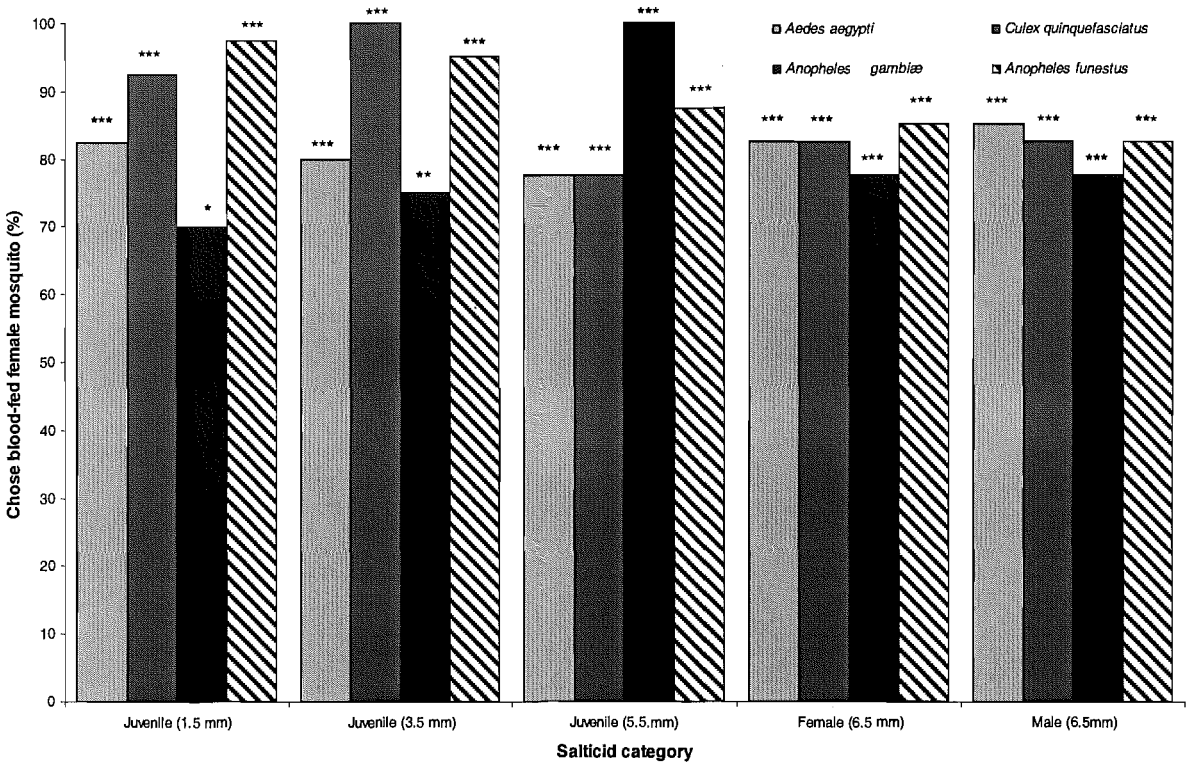

Figure 10. Percentage of times *Evarcha culicivora* (body length and age variable) chose the odour of blood-fed female blood-fed female mosquitoes (*Aedes aegypti, Culex quinquefasciatus, Anopheles gambiæ, Anopheles funestus*) rather than the odour of conspecific male mosquitoes. Test spider had simultaneous access to the odour of two types of prey but no visual cues were available. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01, *P<0.05).

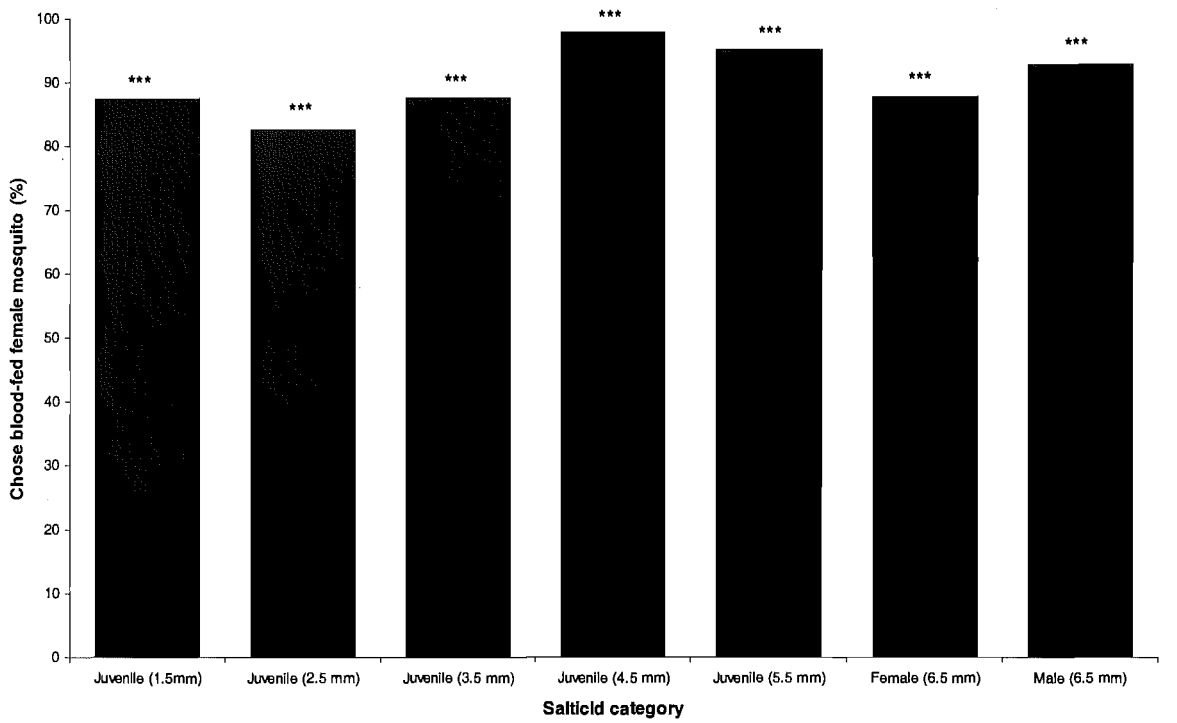

Figure 11. Percentage of times *Evarcha culicivora* (body length and age variable) chose the odour of blood-fed female blood-fed female mosquitoes (*Anopheles gambiæ*) rather than the odour of conspecific female sugar-fed mosquitoes. Test spider had simultaneous access to the odour of two types of prey but no visual cues were available. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001).

Odour-based prey choice using prey that has been immobilised

When the alternative was an immobilised conspecific male mosquito, *E. culicivora* chose immobilised blood-fed female mosquitoes significantly more often than the male mosquitoes. This trend held regardless of the sex-age-size category of the individual of *E. culicivora* being tested (Fig. 12). Pooling data from all these tests, 162 (81%) spiders chose blood-fed female mosquitoes, whereas only 38 (19%) chose the conspecific male mosquitoes.

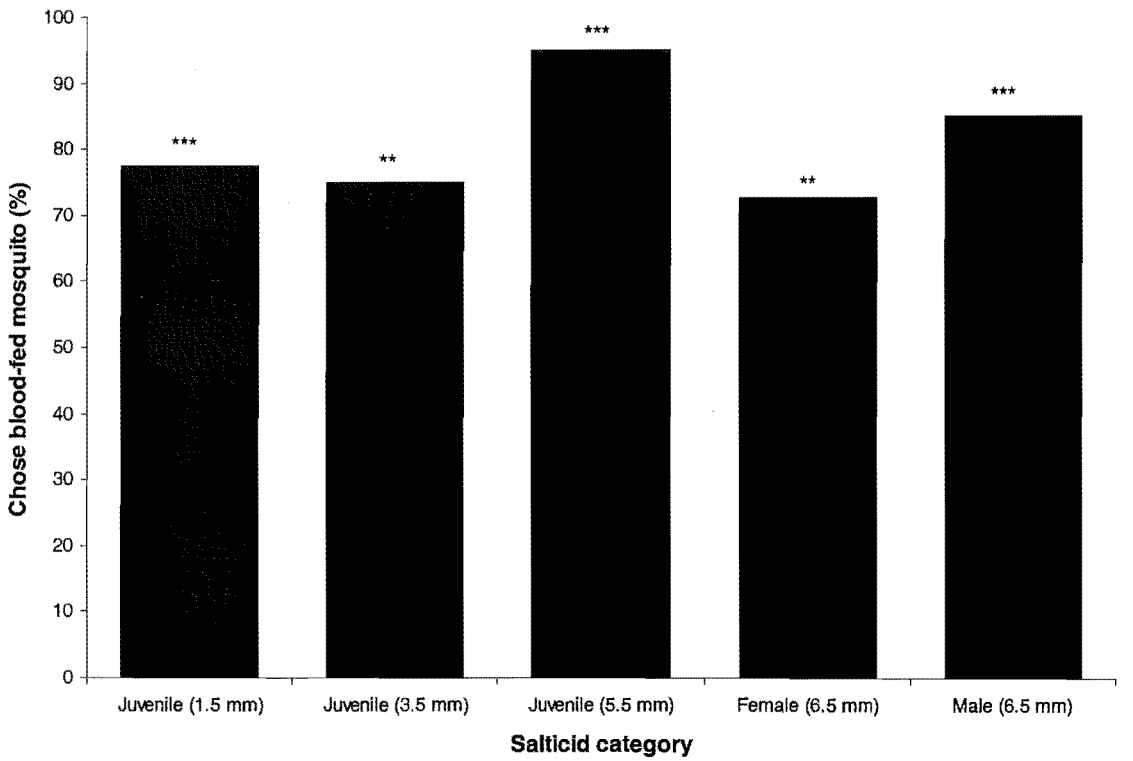

Figure 12. Percentage of times *Evarcha culicivora* (body length and age variable) chose the odour of blood-fed female blood-fed female mosquitoes (*Anopheles gambiæ*) rather than the odour of conspecific male mosquitoes. Test spider had simultaneous access to the odour of two types of prey but no visual nor vibrational cues were available because mosquitoes were immobilised during test. N=40 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01).

Discussion

An earlier study (Wesolowska & Jackson, 2003) demonstrated that female mosquitoes are the most common component of *E. culicivora's* diet in the field, and those data suggested the hypothesis investigated here: that, by means of its prey-choice behaviour, *E. culicivora* feeds indirectly on vertebrate blood. These testing methods in the laboratory provided data on *E. culicivora's* prey-choice decisions independent of potential confounding effects from prey behaviour. In tests using lures, *E. culicivora* consistently chose the blood-carrying female mosquitoes instead of prey that were not carrying blood (sugar-fed female mosquitoes, male mosquitoes and a variety of arthropod species that do not feed on blood). Besides demonstrating a preference for the blood-carrying prey, data from the lure-tests illustrated that *E. culicivora* can identify its preferred prey by sight alone. In tests using an olfactometer, *E. culicivora* consistently chose the blood-carrying female mosquitoes instead of prey that were not carrying blood (sugar-fed female mosquitoes, male mosquitoes and a variety of arthropod species that do not feed on blood). Besides demonstrating a preference for the blood-carrying prey, data from the olfactometer-tests illustrated that *E. culicivora* can identify its preferred prey by odour alone.

A recent study has shown that, after feeding on blood, females of *An. gambiæ* suffer a general reduction in their ability to avoid being captured by salticids (Roitberg *et al.*, 2003), but the mosquito's diminished defensive ability could not be the proximate explanation for the findings presented here because *E. culicivora's* reactions to inanimate lures alone and to odour alone were tested, not *E. culicivora's* reactions to living prey. This is an important distinction, giving me confidence that these data reveal specifically *E. culicivora's* prey-choice decisions rather than the prey's ability to defend itself (i.e., these findings were not directly influenced by the prey's defences).

The salticid species Roitberg *et al.* (2003) used, *Salticus scenicus*, is not known to feed unusually often on mosquitoes in the field, nor was their study designed to test for preferences. However, the findings from their study suggest a hypothesis concerning the adaptive significance of *E. culicivora's* prey-preference: by choosing blood-fed female mosquitoes, *E. culicivora* might be singling out especially easily captured prey. However, other work (Chapters 4, 5, 6) and work in progress (RRJ, pers. comm.) does not support this hypothesis and suggests instead that the blood-meal itself is important to *E. culicivora.*

Evarcha culicivora's prey-choice behaviour appears to be driven by an innate prey-preference for blood-fed female mosquitoes. Having used second and third generation spiders from

laboratory rearing under standardized conditions (see: Roff, 1998), prior experience, maternal effects (Wade, 1998) and other indirect genetic effects (Moore *et al.*, 1997) are unlikely alternative explanations for these findings.

By sight alone, *E. culicivora* also made prey-size choices. When all lures were blood-fed female mosquitoes and when all lures were sugar-fed female mosquitoes, 1.5 mm juveniles of *E. culicivora* chose the smaller lures, but all other size classes of *E. culicivora* chose the larger lures. However, *E. culicivora's* preference for the blood-carrying prey evidently took precedence over size preference. When the size lure that was preferred was a sugar-fed female mosquito, whereas the size that was not preferred was a blood-fed female mosquito, *E. culicivora* chose the blood-fed female mosquito that belonged to the non-preferred size class instead of the sugar-fed female mosquito that belonged to the preferred size class.

These experiments using lures showed that *E. culicivora*, along with araneophagic and myrmecophagic salticids (Li & Jackson, 1996a,b) can identify its preferred prey by sight alone. There has been a persistent trend in the literature to emphasize the role of vision in salticid biology and largely to neglect the role of other modalities (Jackson & Pollard, 1997). Among myrmecophagic salticids, *Habrocestum pulex* can identify ants by odour alone (Clark *et al.*, 2000). Among araneophagic salticids, *Portia fimbriata* from one particular habitat (rain forest in Queensland) can identify by odour alone one of the spiders (*Jacksonoides queenslandicus*) on which it frequently feeds (Jackson *et al.*, 2002). Evidently, *E. culicivora* can also identify its preferred prey by odour alone.

Earlier studies have shown that araneophagic and myrmecophagic salticids have innate prey-preferences for spiders and ants, respectively (Li & Jackson, 1996a). *E. culicivora* is, however, the first predator, salticid or otherwise, that has been shown experimentally to prefer specifically blood-fed female mosquitoes as prey.

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CHAPTER FOUR

Optical cues that govern the prey-choice decisions of a mosquito-eating spider from East Africa, Evarcha culicivora

Abstract

Evarcha culicivora is an East African salticid spider that feeds indirectly on vertebrate blood by choosing, as prey, blood-carrying female mosquitoes, which it can distinguish using vision alone. The optical cues for this prey-choice behaviour were investigated experimentally under standardised conditions in the laboratory. *E. culicivora* was tested with lures made by altering the appearance of dead mosquitoes: features from the head and thorax of one mosquito were combined with features from the abdomen of a different mosquito ('hybridised lures'). *E. culicivora* was also tested by using 3D animation software to create virtual mosquitoes and then altering the prey's size, antennae, and movement. The findings suggest that primary factors influencing the prey-choice decisions of *E. culicivora* include mosquito's size, the shape of its abdomen, and the appearance of its antennae. There also appears to be a hierarchy of criteria: when mosquitoes had engorged abdomens, *E. culicivora* chose mosquitoes with female antennae in preference to otherwise identical mosquitoes with male antennae, but features of the antennae appeared not to be attended to when the abdomens of the mosquitoes were not engorged.

Introduction

A predator's diet is the prey the predator actually eats in nature, and a predator's preference is only one potential influence on diet (Morse, 1980). Preference, a cognitive attribute of the predator, is revealed by the predator's prey-choice decisions (see Chapter 3). The diet of *Evarcha culicivora*, an East African jumping spider (Salticidae), consists mainly of mosquitoes (Wesolowska & Jackson, 2003). Prey-choice experiments have revealed that blood-fed female mosquitoes are *E. culicivora's* preferred prey, and that *E. culicivora* can distinguish blood-fed female mosquitoes from other prey by using visual cues alone and by using olfactory cues alone (Chapter 3).

Most salticids appear to be generalist predators of motile insects (Richman & Jackson, 1992; Foelix, 1996), but there are some remarkable examples of specialised preferences in this family of spiders. In particular, species that prefer other spiders as prey (araneophagic salticids) appear to be especially common in the subfamily Spartaeinae (Harland & Jackson, 2001; Cerveira *et al.*, 2003)

and there are also species, belonging to several subfamilies, that prey especially often on ants (Edwards *et al.*, 1974; Cutler, 1980; Li & Jackson, 1996). Salticids have unique, complex eyes and high visual resolving power (Land, 1969a,b; Land, 1974, Williams & McIntyre, 1980). Salticids have an outstanding ability to discriminate, by sight alone, between different prey types that, on the whole, appear to people to be quite similar to each other. Among araneophagic salticids, *Portia fimbriata* and *P. labiata* are especially striking examples. *P. fimbriata* from Queensland appears to be unique because it has special tactics for preying on salticid spiders (Harland & Jackson, 2001) and *P. labiata*, from Los Baños in the Philippines, has special tactics for preying on an araneophagic spitting spider, *Scytodes pallida* (Jackson *et al.*, 1998; Li & Jackson, 2003). In laboratory experiments, *P. fimbriata* and *P. labiata* identified these specific types of prey by vision alone. The specificity of *E. culicivora's* predatory behaviour rivals that of *Portia*. Earlier studies (Chapter 3) have shown that *E. culicivora* can identify its preferred prey (blood-carrying female mosquitoes) by vision alone. This chapter is an experimental study aimed at clarifying the optical cues that govern the prey-choice decisions of *E. culicivora*. Using lures made from dead prey and lures made with 3D animation software (virtual lures), I test specifically for the roles of movement, body size, abdominal shape (i.e., whether the abdomen was engorged or slim) and the appearance of the antennae (i.e., antennae from male versus female mosquitoes).

The most noticeable difference, to the human eye, between blood-fed female mosquitoes and non-blood fed female mosquitoes is that the former has a rotund, red abdomen, whereas the latter has a dark, elongated abdomen. The abdomens of male mosquitoes and of female mosquitoes that have not fed on blood are similar, but, once a person has become skilled at detecting the difference, the antennae of male and female mosquitoes differ considerably in appearance. The antennae of male mosquitoes have a plumose appearance because of the presence copious whorls of setae (Chapter 2). The female's antennae, in contrast, appear almost naked. In this chapter, I investigate whether these same features that people use for distinguishing between different mosquitoes are also used by *E. culicivora*.

Materials and Methods

Testing with hybrid lures

This study was conducted at the International Centre of Insect Physiology and Ecology (ICIPE) Mbita Point Field Station (MPFS) in south-western Kenya. All living spiders came from a laboratory culture, and standard spider-laboratory procedures were adopted (Jackson & Hallas,

1986). Methods for making lures were, in general, the same as described in Chapter 3 except that, in these experiments, some lures were 'hybridised'.

Hybrid lures were made by separating the abdomens from the heads and thoraces of mosquitoes and then gluing anatomical portions of separate mosquitoes together on cork discs. Lures were made from males, sugar-fed females and blood-fed females of *Anopheles gambiæ* (for details concerning diet, see Chapter 3). Two hybridised lures were made: (1) the abdomen of a blood-fed female *An. gambiæ* was glued onto the head and thorax of a male *An. gambiæ*; (2) the abdomen of a male *An. gambiæ* was glued onto the head and thorax of a female *An. gambiæ*. These hybrid lures provided the spider with potential cues from the male and from the female. In addition, 'intact' lures were made from males, sugar-fed females and blood-fed females of *An. gambiæ*.

Each individual test spider was given simultaneous access to two types of lures (each lure was placed at one of the two ends of a Y-shaped ramp angled up at 20°; Fig. 1). Methods for simultaneous-presentation tests using lures were as in previous salticid studies (Li & Jackson, 1996) except for some variations introduced for facilitating the testing of juvenile spiders (previous studies have been primarily with adults). Instead of using motionless lures, during these tests the lures moved. This modification of the methods was introduced because preliminary testing indicated that juveniles of *E. culicivora* were disinclined to respond to motionless lures. Another modification was that the testing ramp was smaller than ramps used in previous studies using adults. These smaller ramps ensured that the test spiders would not be further than 20 body lengths away from the lure at the beginning of the test, 20 body lengths being the distance other studies have established as the distance at which discrimination between prey and non-prey is readily achieved (Harland *et al.*, 1999).

Behind the lures at the end of each arm of the ramp, a rectangular piece of wood called the 'wall' (15 mm wide, 15 mm high and 3 mm thick) was glued, serving as the background against which *E. culicivora* saw the lure. Centred at the end of each arm there was an oblong hole (20 mm long and about 4 mm wide; about 5 mm from the rectangular wall). Lures were placed on top of this hole and were attached to pins that could be used for manually manipulating the lure's movement from under the ramp. Before testing began, and at 3 min intervals throughout the test, lures were moved (rotated) for *c.* 10 s. Lures were rotated by slowly spinning the pins that were stuck in the cork bases of each lure so that lures made one full rotation every 2-3 s. After each movement period the lure was positioned so that the mosquito was angled at about 45° from the base of the ramp. In this position the spider approaching the lure could see the lure's head as well as its abdomen.

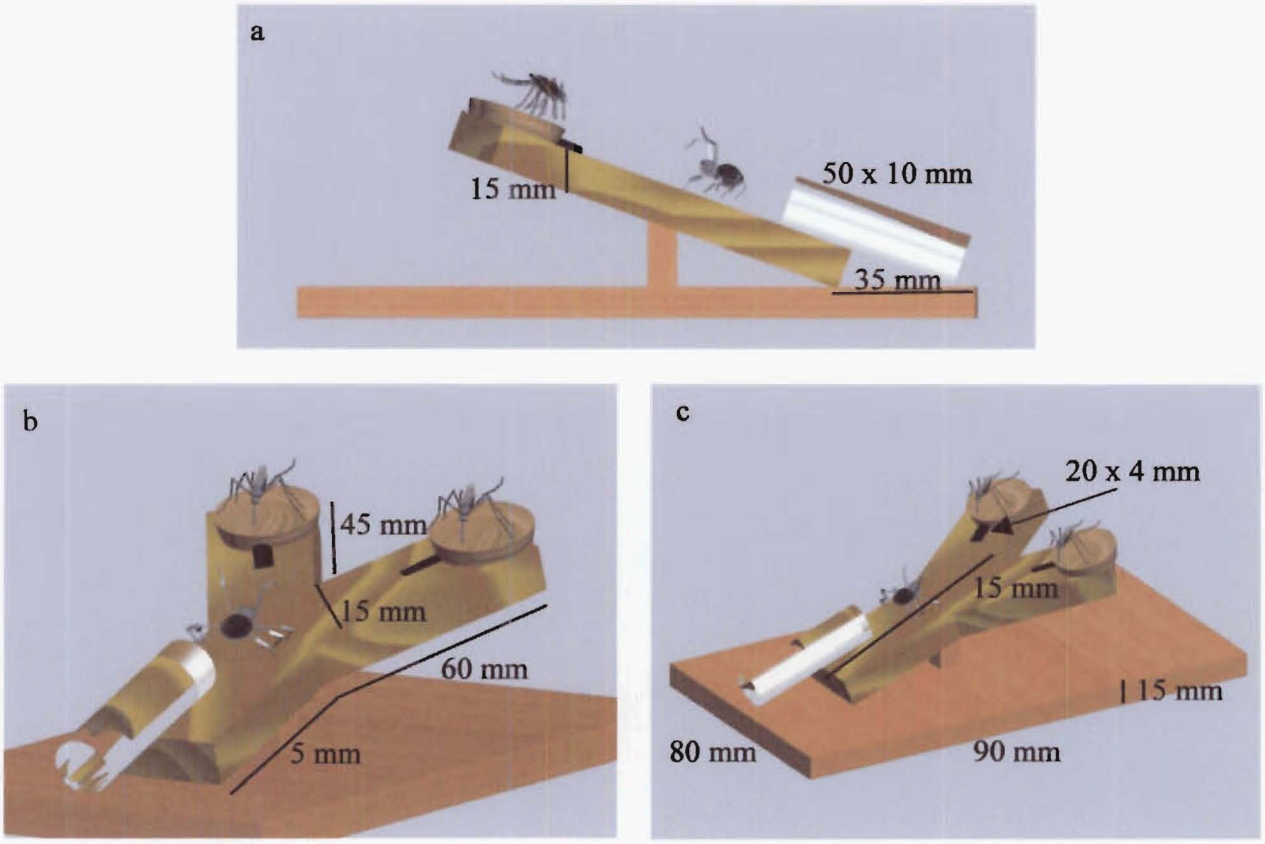

Figure 1. Ramp (not drawn to scale) used in experiments with hybrid lures. Wooden wall at end of each arm not shown. **a)** Side view: showing plastic vial from which spiders began the tests. **b)** Perspective view: showing choices of lures and basic dimensions. **c)** Perspective view: showing oblong holes under each lure to place pins (attached to the cork discs) with which to move lures.

A plastic vial (50 mm long X 10 mm diameter) (packed with cotton to about 5 mm from the open end) was placed on the stem of the ramp. Tests began when a spider, which had been placed on the cotton within the vial, walked out of the vial and on to the ramp. After each test, the ramp was wiped with 80% ethanol and the cotton in the vial was changed to eliminate chemical cues from draglines of previously tested spiders that might have affected the outcome of subsequent tests.

All spiders used in tests were fourth instar (i.e., third instar post-dispersal from the brood sac) *E. culicivora* juveniles (body length, *c.* 3.5 mm) that had been subjected to a fast of 5-7 days before testing. No test spider was used more than once.

Unsuccessful tests were those in which the test spiders walked down the ramp (away from the lure), tests in which 20 min elapsed without the test spiders walking toward a lure, and tests in which the tests spiders jumped off the ramp or failed to move. Unsuccessful tests were infrequent. Successful tests were those in which spiders walked up the ramp toward one or the other lure (usually it was obvious that the test spider was stalking and watching the lure) and stayed on one of the two arms of the ramp for longer than 30 s at a distance of no more than 10 mm from the lure and when the test spider jumped on one of the lures.

Results were analysed using chi-square tests for goodness of fit (null hypothesis: the two choices are made equally often) (Sokal & Rohlf, 1995).

Testing with virtual prey

The study was conducted at the Spider Laboratory at the University of Canterbury in Christchurch, New Zealand. Laboratory protocol was the same as described above for prey-choice tests using hybrid lures.

All spiders used in tests had been starved for 5-7 days before testing. Tests were carried out using adult males and females of *E. culicivora* (body lengths, 4.5 and 5.5 mm) as well as small juveniles of *E. culicivora* (body length, 1.5 mm). Results with males and females of *E. culicivora*, being statistically indistinguishable, were pooled.

Spiders were presented with virtual mosquitoes (for drawing and animation methods, see Appendix I) projected (refresh rate of computer monitor 60 Hz) at a screen resolution of 800 x 600 pixels using a Telex P400 LCD data projector (Fig. 2). The projected image was reduced with the aid of a magnifying lens in front of the projector's lens. This had the (counterintuitive) effect of reducing the projected image. The size of the projected virtual mosquitoes was calibrated by varying the size of the window in which the animation was playing on the computer (until the mosquitoes, in

reduced form, measured 3.2 mm on the screen onto which they were projected). The image was projected onto a fine-ground matte unmarked type D Nikon F3 focusing screen (39 mm wide X 30 mm high), located at a distance of *c.* 150 mm from the projector lens. The array of lenses and the focusing screen were set up on magnetic clamps on a steel platform to prevent movement of the components. The projector was positioned at an angle of *c.* 10°, pointing down, and the testing ramp (a stainless steel ruler, 15 mm wide X 150 mm long) was angled up *c.* 25° toward the focusing screen. With these angles shadows were avoided and the stalking spiders did not obscure the projected image. The ramp was held in place with a clamp. When juvenile spiders were tested, the end of the ramp was placed 2 mm in front of the screen. When adults were tested, the end of the ramp was placed 5 mm in front of the screen. This separation between the ramp and the screen ensured that tests spiders did not simply walk on the screen and maximised the chances of spiders leaping on the virtual prey. Having a ruler as a ramp ensured that distance from the screen could be measured accurately.

Virtual mosquitoes (body length, 3.2 mm) were projected side-on so that potential cues from the head as well as from the abdomen were visible to spiders. Virtual mosquitoes were derived from blood-fed females of *Anopheles* and were animated to be grooming intermittently (for details, see Appendix I). Because results from another study (Chapter 7) showed that *E. culicivora* chooses virtual mosquitoes more often when the mosquito's abdomen is red than when it is black, the abdomens were always red and the heads and thoraces were in 'greyscale' (black and white). During tests, two virtual mosquitoes that differed only in one respect were placed side by side. Which virtual mosquito was on the left-hand side of the screen or on the right-hand side of the screen was randomised (animations were made for both sides of the screen).

Three choice tests were carried out: (1) the antennae of the virtual mosquitoes were different (one mosquito had male antennae and the other had female antennae); (2) the behaviour of the mosquitoes differed (both lures moved simultaneously; one mosquito was presented grooming naturally and the other mosquito jumped, intermittently, i.e., movement was simultaneous but not continuous; and (3) both mosquitoes were physically and behaviourally identical, but one had been scaled down to 75% of the size of the other. Animation of grooming was based on frame-by frame analysis of digital video footage of grooming behaviour, see Appendix I.

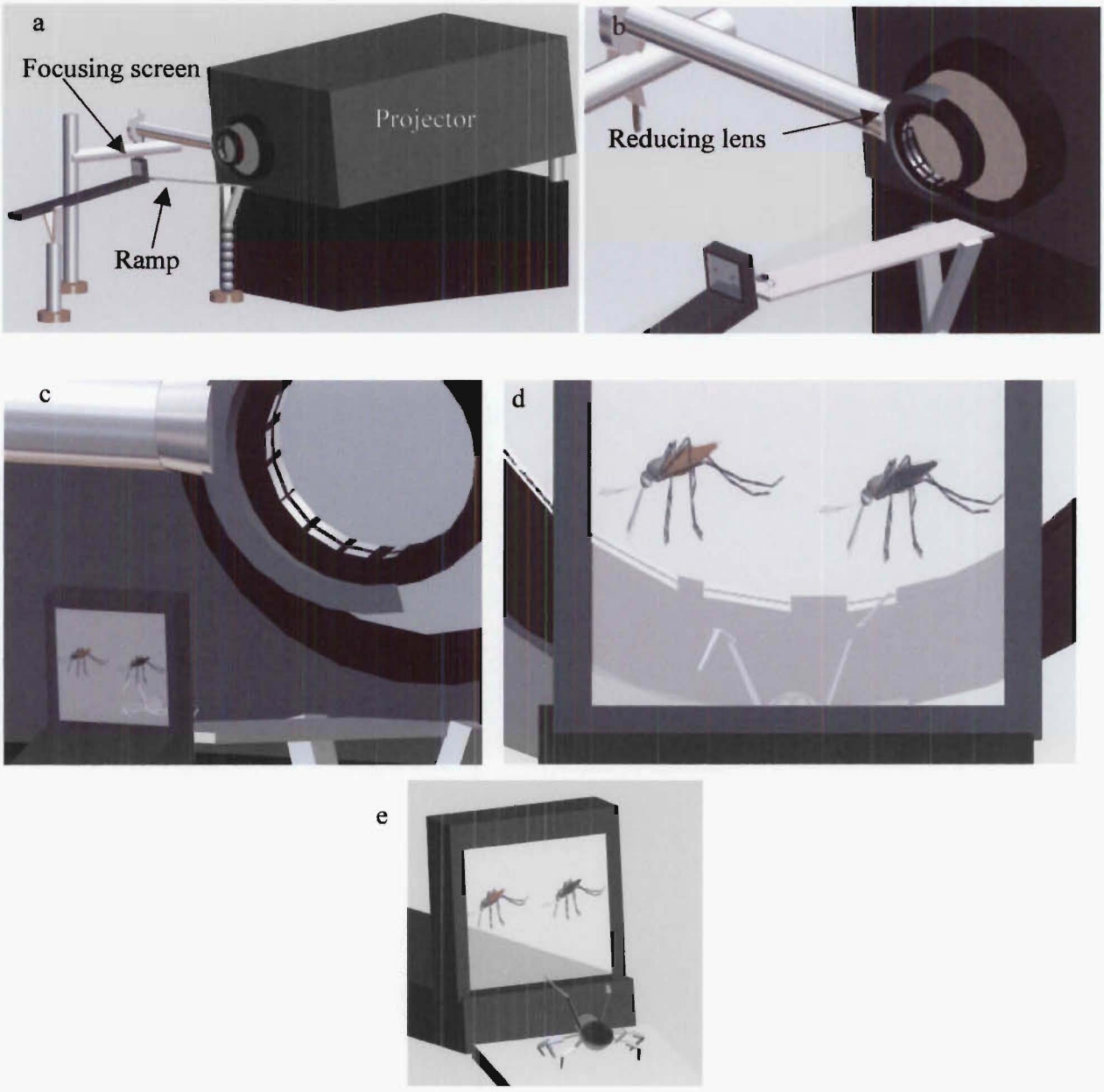

Figure 2. Methods used for presenting spiders with animated virtual prey. **a)** Virtual prey experimental set-up (magnetic steel platform on which ramps were placed not shown): a data projector projecting animation through reducing lens on to a focusing screen. Spiders approach focusing screen on a ramp between the projector and the screen. **b)** Close-up view of spider on ramp looking at virtual prey projected through reducing lens onto focusing screen. **c)** Enlarged view of spider looking at virtual mosquitoes with 'red' and 'black' abdomen (Chapter 7) (reducing screen seen in background). **d)** Spider seen from behind the focusing screen watching virtual mosquitoes. **e)** Appearance of virtual mosquitoes on focusing screen. Spider seen from in front of screen. Note: spider not to scale.

Adult test spiders were placed in a small piece of transparent PVC tubing (10 mm long, inner diameter 8 mm) corked at both ends and stuck to the ramp with Blu Tac 50 mm from the end of the ramp. When adults were used as test spiders, testing began when the cork that faced the focusing screen was removed from the tubing, allowing the spider to leave. Juveniles were placed 10 mm from the end of the ramp using a soft-haired paintbrush. The spider walked on to the tip of the brush which was then positioned by the ramp. This was not difficult; *E. culicivora* is a 'calm' salticid that will sit peacefully on a brush. This different method for beginning tests was chosen for juveniles because, at 50 mm from the screen, the virtual prey was probably too far away for the spiders to identify (see Harland *et al.*, 1999) and, at 10 mm, the tubing cast a shadow on the animated mosquitoes. Because of the differences in how tests began with adults and juveniles, tests with juveniles began as soon as the spider walked off the brush and on to the ramp.

Tests with virtual lures lasted 15 min. However, if the test spider had begun stalking, tests were extended until the end of the stalking bout (this was never more than 18 min). Stalking is defined as the behaviour of the spider once it had oriented toward the prey and had begun approaching in a direct line while it waving its palps (see Chapter 6). Stalking, being 'purposeful' in appearance, was a distinctive behaviour and readily identifiable. The stalking spider tended to slow down as it neared the lure.

Successful tests were those in which the spider jumped and landed directly on a mosquito, as well as those in which the spider stalked a virtual mosquito and got to the end of the ramp and stayed there, oriented toward the mosquito, for a period of 30 s. The latter usually happened with juvenile, not adult, spiders. However, even when juvenile spiders did not jump on the virtual prey, they often raised legs I. This action is typical pre-jump behaviour for *E. culicivora.*

Unsuccessful tests were those in which spiders walked down the ramp (away from the projected image), tests which lasted longer than 15 min without the test spider stalking the virtual prey, and tests in which the test spider jumped off the ramp or failed to move. If a test was unsuccessful, the same spider was sometimes used for the same test on the same day (at least 60 min later). However, spiders were never tested more than twice on any given day.

Results of prey-choice tests were analysed using chi-square tests for goodness of fit (null hypothesis: the two choices are made equally often). Analysis of variance (ANOVA) was carried out to determine whether the choice of prey affected the times at which prey-discrimination was made (i.e., the time at which test spiders began to stalk each type of prey). Latencies to orient toward, begin stalking and jump on specific virtual prey were compared using ANOVA. This analysis was

also carried out to determine whether the choice of prey affected the distance at which prey-discrimination was made (i.e., the distance at which spiders began to stalk each type of prey). Distances from which spiders oriented toward, began stalking and jumped on specific virtual prey were compared using ANOVA (Sokal & Rohlf, 1995).

Results

Testing with hybrid lures

Test spiders chose the lure made from a blood-fed female *An. gambiæ* significantly more often than they chose the lure made from the abdomen of a blood-fed female and the head and thorax of a male *An. gambiæ* (χ^2=5.667; P<0.05; N=51) (Fig. 3). However, there was no significant difference in the number of spiders that chose lures made from the head and thorax of a female *An. gambiæ* that were attached to the abdomen of a male *An. gambiæ* and the number of spiders that chose lures made from an intact male *An. gambiæ* (χ^2=2.4; NS; N=60) (Fig. 3).

For mosquito lures on which the head and thorax were identical, the shape of the abdomen appeared to determine *E. culicivora's* prey-choice behaviour. When the lure's abdomen was engorged, it was chosen more often than when the lure's abdomen was thin (Fig. 3). Test spiders chose lures made from blood-fed females of *An. gambiæ* significantly more often than they chose lures made from the head and thorax of a female *An. gambiæ* attached to the abdomen of a male *An. gambiæ* (χ^2=7.475; P<0.01; N=59). *E. culicivora* also chose the lure made from a male's head and thorax attached to the abdomen of a blood-fed female more often than they chose the lure made from an intact male *An. gambiæ* (χ^2=9.308; P<0.01; N=52).

When cues from the head and thorax were the same, but the abdomen of neither lure was engorged, there was no significant difference in how the test spiders reacted to the lures. *E. culicivora's* response to lures made from the head and thorax of a female *Anopheles gambiæ* attached to the abdomen of a male *An. gambiæ* did not differ from its response to lures of sugar-fed females (χ^2=0.074; NS; N=54) (Fig. 3).

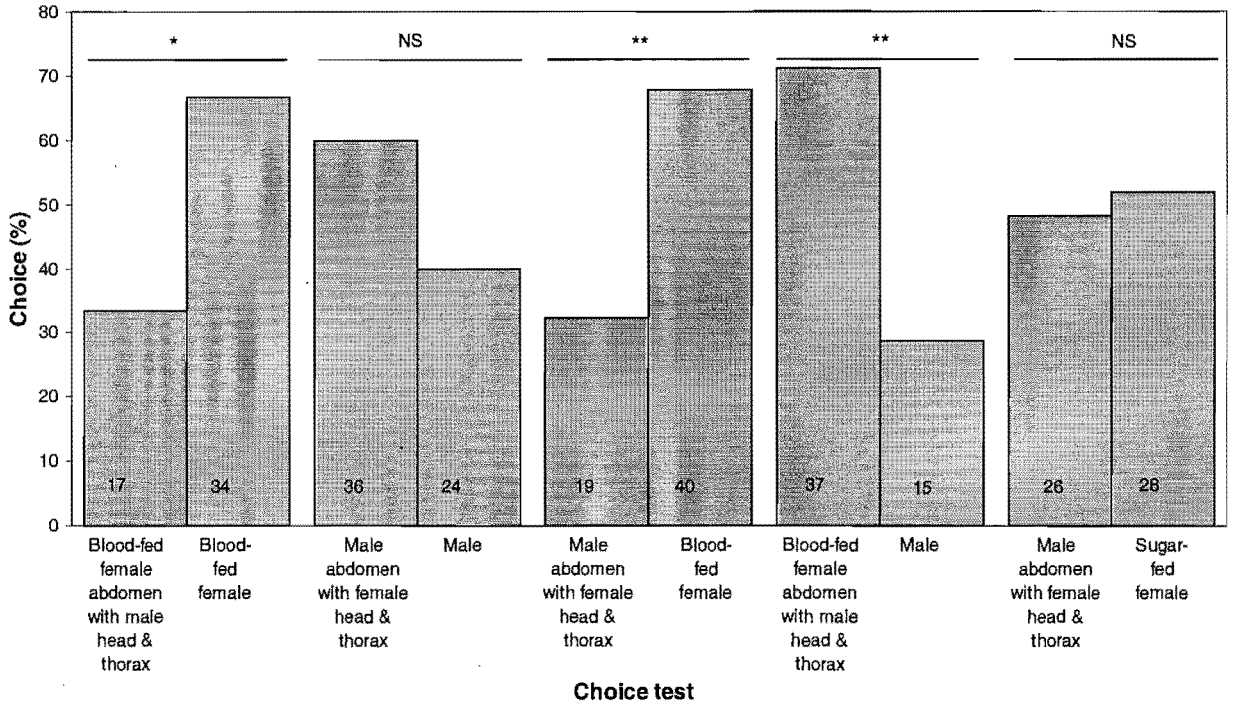

Figure 3. Percentage of times fourth instar *Evarcha culicivora* (body length, *c.* 3.5 mm) chose different prey on the basis of visual cues alone. Test spider had simultaneous access to two lures made from *Anopheles gambiæ* mosquitoes. Intact lures: blood-fed female *An. gambiæ*; sugar-fed male *An. gambiæ*; sugar-fed female *An. gambiæ*. Hybrid lures: abdomen from blood-fed female *An. gambiæ* with head and thorax from male *An. gambiæ;* abdomen from sugar-fed male *An. gambiæ* with head and thorax from female *An. gambiæ*. Numbers of spiders that chose each prey shown in bars. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often, **P<0.01, *P<0.05).

Testing with virtual prey

Small juveniles of *E. culicivora* (body length, 1.5 mm) attended to cues from the antennae of prey, choosing virtual prey with female antennae significantly more often (χ^2=6.545, P=0.01, N=22) than virtual prey with male antennae that were otherwise identical (Fig. 4). The same result was obtained with adults, 75% (n=15) of which chose the virtual prey with female antennae instead of the virtual prey with male antennae (n=5) (χ^2=5, P<0.05, N=20). That the results were comparable for adults and small juveniles provided confidence that the results from subsequent tests with juveniles were valid, and subsequently only 1.5 mm spiders were tested.

Naturally moving (grooming) virtual mosquitoes were chosen significantly more often (χ^2=6.545, P=0.01, N=22) than virtual prey that moved in an odd manner ('jumped') (Fig. 4). When virtual prey were identical in all respects except that one had been scaled down to 75% of the size of the other, juveniles of *E. culicivora* chose the smaller of the two virtual prey significantly more often (χ^2=10.667, P<0.01, N=24) than the larger prey (Fig. 4).

When comparisons were made between the choice of prey and the time to orient towards it, the time to begin to stalk it, and the time to attack the prey, there was no statistically discernible effect of the choice of prey (ANOVA). There was no evidence that prey-choice affected the distance from which spiders oriented toward, began stalking or jumped on prey (ANOVA).

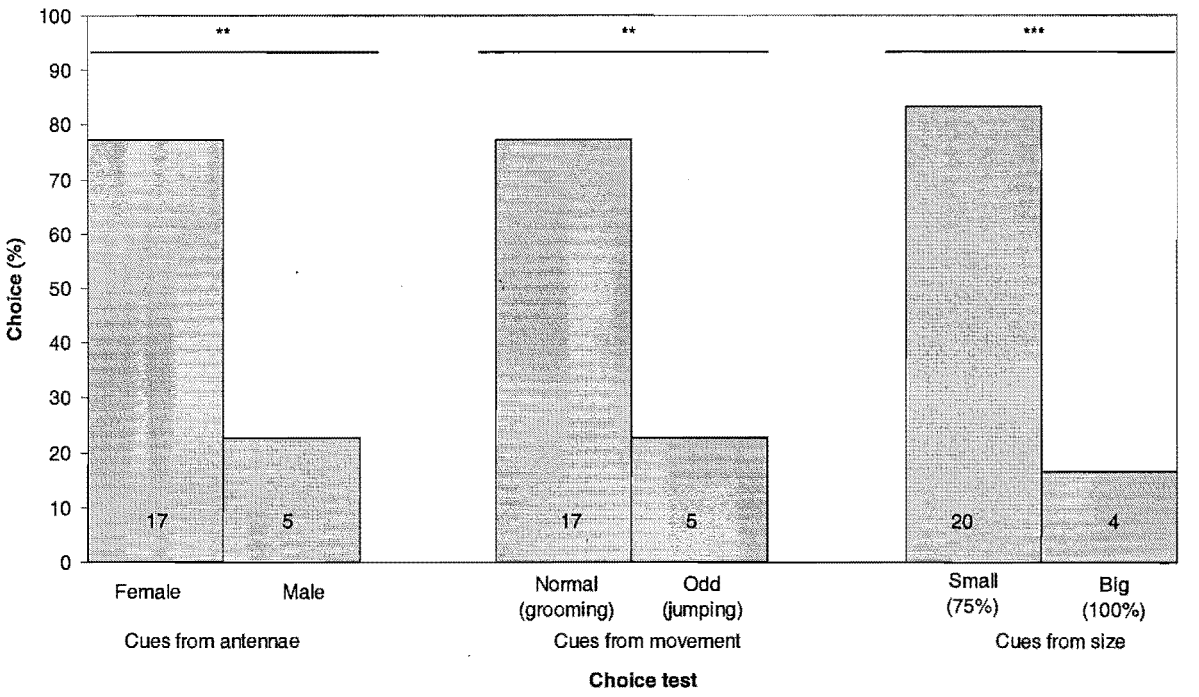

Figure 4. Percentage of times juveniles of *Evarcha culicivora* (body length, 1.5 mm) chose different virtual prey on the basis of visual cues alone.. Test spider had simultaneous access to two virtual lures based on blood-fed females of *Anopheles gambiæ* mosquitoes. All choice tests with identical mosquitoes that differed in one cue: antennae (One prey: male antennae. Other prey: female antennae); movement (One prey: intermittently grooming. Other prey: intermittently jumping); Size (One prey: life-size, 3.2 mm long. Other prey: same mosquito but 25% smaller). Numbers of spiders that chose each prey shown in bars. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often, *** $P<0.001$, ** $P<0.01$).

Discussion

Earlier work (Chapter 3) showed that, when using vision alone, *Evarcha culicivora* can identify its preferred prey (blood-fed female mosquitoes). The work presented in this chapter clarifies the optical cues by which *E. culicivora* identifies its prey. By using lures and by using virtual prey, I varied visual cues in the absence of variables related to odour, sound or substrate-vibration. Prey movement was also controlled for. The only potential prey-identification cue available for *E. culicivora* in these experiments were from the prey's appearance. With virtual mosquitoes, in particular, prey appearance could be varied precisely while keeping other features of the prey strictly constant.

Evidently, *E. culicivora* attends to the behaviour of the prey, more often choosing mosquitoes that groomed in a natural manner instead of mosquitoes that jumped. (The fact that five spiders did choose the jumping mosquito suggests that the spiders were not simply choosing the alternative because the jumping mosquito was too 'scary'.)

The engorged abdomens of female mosquitoes (male mosquitoes do not feed on blood, see Chapter 2 for details) are an easily-detected visual indicator of the presence of blood and *E. culicivora* chose the lures with engorged abdomens in preference to lures with thin abdomens, regardless of other cues. Cues from the shape of the abdomen apparently take priority over cues from the head. However, *E. culicivora* can discriminate between male and female mosquitoes on the basis of their antennae, as people do. There seems to be an interesting parallel here between how people and the spider see mosquitoes. (A person trained to sex mosquitoes normally relies on the appearance of antennae). When both lures had engorged abdomens, *E. culicivora* chose lures with female heads and thoraces in preference to lures with male heads and thoraces, indicating that they do, also, attend to cues from the head and thorax of mosquitoes, at least when the shape of the abdomen is appropriate for a blood-carrying female. Data from using lures made from dead mosquitoes and from using virtual mosquitoes both implied that the appearance of the antennae mattered to spiders ranging from 1.5-5.5 mm long.

Cues from the antennae may require especially careful processing with high-acuity eyes, whereas the shape of the abdomen may be more easily determined by the spider, quickly informing it of the presence or absence of the blood that it is after. Perhaps cues from the antennae are especially important when the abdomen of a mosquito is not visible (frontal view), but this was not tested. Nevertheless, that 1.5 mm individuals of *E. culicivora* could differentiate virtual lures on the

basis on the antennae alone illustrates remarkable ability for form perception by a minute spider with tiny anterior-median eyes (see Appendix II).

Given that only female mosquitoes feed on blood, it is somewhat paradoxical that the results presented in this chapter suggest that, when the cues from the abdomen are from thin (not blood-engorged) abdomens, *E. culicivora* does not attend to cues from the heads and thoraces of mosquitoes. Perhaps, for *E. culicivora*, whether the prey is a male mosquito or a female mosquito does not matter when the cue for blood (engorged abdomen) is absent. However, this is not an entirely satisfactory explanation because the presence of blood necessarily implies that the mosquito is female, yet *E. culicivora* attends to cues from the head when both lures are engorged. Further tests in which non-engorged virtual mosquitoes that have different antennae are presented to spiders may help resolve this seeming paradox.

That blood may be an important nutrient for *E. culicivora* has not yet been fully established, but preliminary evidence (XJN & RRJ, unpublished data) suggests that it is. The evidence presented in this chapter provides support for the hypothesis that the presence of blood in prey is the primary factor in *E. culicivora's* prey-choice behaviour. In these tests, lures with engorged abdomens were preferred to any alternative. However, *E. culicivora's* prey-choice behaviour is complex, with several other factors apparently influencing *E. culicivora's* prey-choice decisions. For example, this spider makes predatory decisions based on prey size (Chapter 3), with smaller juveniles of *E. culicivora* choosing smaller prey. Tests with virtual mosquitoes provided especially strong confirmation of this size preference because in all other respects the virtual mosquitoes were identical (i.e., there were no species-related confounding effects). Contrary to popular portrayal of spider behaviour, *E. culicivora* certainly does not go for "any old prey as long as it's alive". *E. culicivora* appears to be a spider that makes fine-discriminations between prey. Perhaps the well-known salticid *Portia* has met its match.

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CHAPTER FIVE

Choice of Anopheles *by* Evarcha culicivora, *a mosquito-eating spider from East Africa*

Abstract

Earlier research showing that *Evarcha culicivora*, an East African salticid spider, feeds indirectly on vertebrate blood by choosing, as prey, blood-carrying female mosquitoes, is extended by experimentally examining the particular genus of mosquitoes chosen by the spider. Prior feeding of all test spiders was standardised (7-day fast). Adults and large juveniles of *E. culicivora* showed no mosquito-genus preferences. However, smaller size classes of *E. culicivora*, when relying on sight alone, chose as prey the blood-fed females of a particular mosquito genus, *Anopheles*, significantly more often than they chose blood-fed female mosquitoes from the genera *Aedes* and *Culex*. Details concerning the optical cues the small juveniles use to distinguish anopheline mosquitoes were studied using virtual prey made with 3D animation software. Resting posture of the mosquito appears to be a primary cue by which the spider identifies *Anopheles*. This is the first report of any predator selecting *Anopheles*, the vector of human malaria, as preferred prey.

Introduction

Evarcha culicivora, a salticid spider from East Africa, can identify its preferred prey by vision alone and by olfaction alone (Chapter 3). *E. culicivora* feeds indirectly on vertebrate blood by choosing blood-fed female mosquitoes as preferred prey. The objective in this chapter is to investigate whether *E. culicivora* discriminates between *Anopheles* and other mosquito genera. Findings from preliminary studies using living prey suggested that the smaller juveniles of *E. culicivora* are predisposed to prey on *Anopheles* in preference to mosquitoes belonging to genera *Aedes* and *Culex*. Here I follow up the preliminary work by testing with lures and by using virtual prey (computer animation).

The possibility that *E. culicivora* targets *Anopheles* as preferred prey is of exceptional interest because mosquito species that serve as vectors for human malaria all belong to this genus (Gillet, 1971). The adults of *Anopheles* have a characteristic resting posture: hind legs raised and abdomen angled up at about 45° from the surface on which the mosquito is standing, with the abdomen forming a straight line with the proboscis. *Aedes* and *Culex*, in contrast, rest with their

abdomens held parallel to the substrate and their heads tilted toward the substrate. In this chapter, besides showing that *E. culicivora* has a genus-specific preference for *Anopheles*, I provide experimental evidence that *E. culicivora* uses posture as an *Anopheles* identification cue.

Materials and Methods

Testing with lures

This study was conducted at the International Centre of Insect Physiology and Ecology (ICIPE) Mbita Point Field Station in south-western Kenya. All living spiders came from laboratory culture, and standard spider-laboratory procedures were adopted (Jackson & Hallas, 1986). All testing was carried out between 0700 h and 1900 h (laboratory photoperiod 12L:12D, lights on at 0700).

A representative species from each of three mosquito genera was used for making lures in simultaneous-presentation tests of prey-choice behaviour. Tests were carried out using laboratory-reared *Anopheles gambiæ* (sensu stricto) (from a colony established from wild gravid females collected at Mbita Point in February 2000), *Aedes aegypti*, and *Culex quinquefasciatus* (the latter two were collected as larvae and reared to adulthood at Mbita Point). Only 'blood-fed' female mosquitoes were used as lures (for details, see Chapter 3). Methods for making lures were the same as those used in Chapter 3 and will not be repeated here.

In order to control for the effect of prey-size on the predatory behaviour of *E. culicivora* (Chapter 3), the mosquitoes chosen for lures were matched for body length. *Anopheles gambiæ* (4.5 mm & 5 mm) was paired with *Aedes aegypti* (5 mm) and *C. quinquefasciatus* (4.5 mm), respectively.

All test spiders had been subjected to a fast of 7 days before testing. Tests were carried out using discrete size classes (body length measured accurately to the nearest 0.5 mm) (body length of juveniles: 1.5 mm, 2.5 mm, 3.5 mm, 4.5 mm, 5.5 mm; body length of adult males and females: 6.5 mm). Juveniles were classed as 'small juveniles' (body length: 1.5 mm-3.5 mm) and 'large juveniles' (body length: 4.5 and 5.5.mm). No test spider was used more than once.

Criteria for successful tests were the same as those adopted in Chapter 3. Data were analyzed using chi-square tests for goodness of fit (null hypothesis: the two choices are made equally often) (Sokal & Rohlf, 1995).

Testing with virtual prey

The study was conducted at the Spider Laboratory at the University of Canterbury in Christchurch, New Zealand. Laboratory protocol was the same as described above for prey-choice tests using lures.

All test spiders had been subjected to a fast of 5-7 days before testing. Tests were carried by presenting juveniles of *E. culicivora* (1.5 mm) with animated virtual mosquitoes (for drawing and animation methods, see Appendix I). Methods for projecting animations (Fig. 1) were the same as those described in Chapter 4 and only details specific to this study are mentioned here.

Animations were based on blood-fed (engorged) female *Anopheles* mosquitoes. Results from another study (Chapter 7) indicated that *E. culicivora* chose virtual mosquitoes more often when the mosquito's abdomen was red than when it was black. Consequently, for these tests, the heads and thoraces of both virtual mosquitoes were in 'greyscale' (black and white) and the abdomens were red. For these tests, the two virtual mosquitoes were identical in all respects except for their posture. One mosquito was placed in a typical *Anopheles* resting posture (body tilted at an angle) while the other mosquito was placed in the posture typical of mosquitoes from genera other than *Anopheles* (body held parallel to the substrate).

E. culicivora is a 'calm' salticid that will sit peacefully on a paintbrush. Spiders were placed 10 mm from the end of the ramp using a soft-haired paintbrush. The spider walked on to the tip of the brush which was then positioned by the ramp. Tests began as soon as the spider walked off the brush and on to the ramp. Tests lasted 15 min but, if stalking had been initiated, tests were extended until the end of the stalking bout (never more than 18 min). Successful tests were those in which the spider jumped and landed directly on a mosquito (i.e., when it apparently made an attempt to capture the virtual prey), as well as those in which the spider stalked a virtual mosquito and got to the end of the ramp and stayed there, watching the mosquito, for a period of 30 s. However, even when juvenile spiders did not jump on the virtual prey, they usually raised legs I as if beginning a jump but then did not complete the manoeuvre.

Tests judged unsuccessful were those in which spiders walked down the ramp (away from the projected image), tests in which 15 min elapsed without the spider stalking prey (see Chapter 4 for definition of 'stalking'), tests in which spiders jumped off the ramp or tests in which spiders failed to move for 15 min. No test spider was used more than once.

Data were analyzed using chi-square tests for goodness of fit (null hypothesis: the two choices are made equally often) (Sokal & Rohlf, 1995).

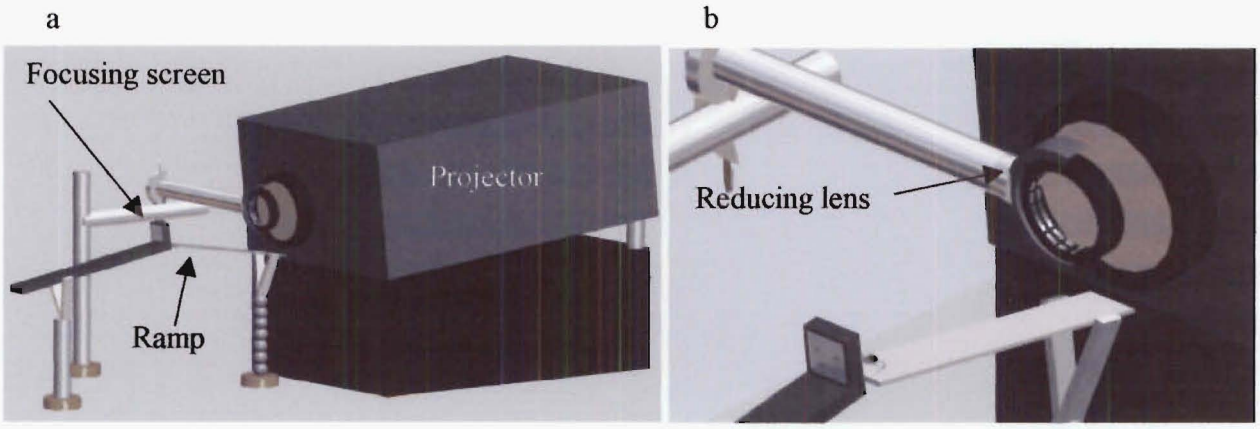

Figure 1. Animation methods. **a)** Virtual prey experimental set-up (magnetic steel platform on which ramps were placed not shown). **b)** Close-up view of spider on ramp observing virtual prey projected through reducing lens onto focusing screen. Note: spider not to scale.

Results

Testing with lures

When prey were matched for size, how often adults (6.5 mm) and large juveniles (4.5 and 5.5 mm) chose *Anopheles gambiæ* was not significantly different from how often they chose *Aedes aegypti* or *Culex quinquefasciatus*. However, small juveniles (1.5–3.5 mm) chose *An. gambiæ* significantly more often than they chose *Aedes aegypti* and *Culex quinquefasciatus* (Fig. 2).

Testing with virtual prey

Small juveniles of *E. culicivora* (1.5 mm) chose the virtual mosquito resting in an *Anopheles* posture (body axis on a 45°) significantly more often (n=18) than an identical virtual mosquito resting in a non-anopheline posture (body horizontal) (n=2) (χ^2=12.88, P<0.001, N=20).

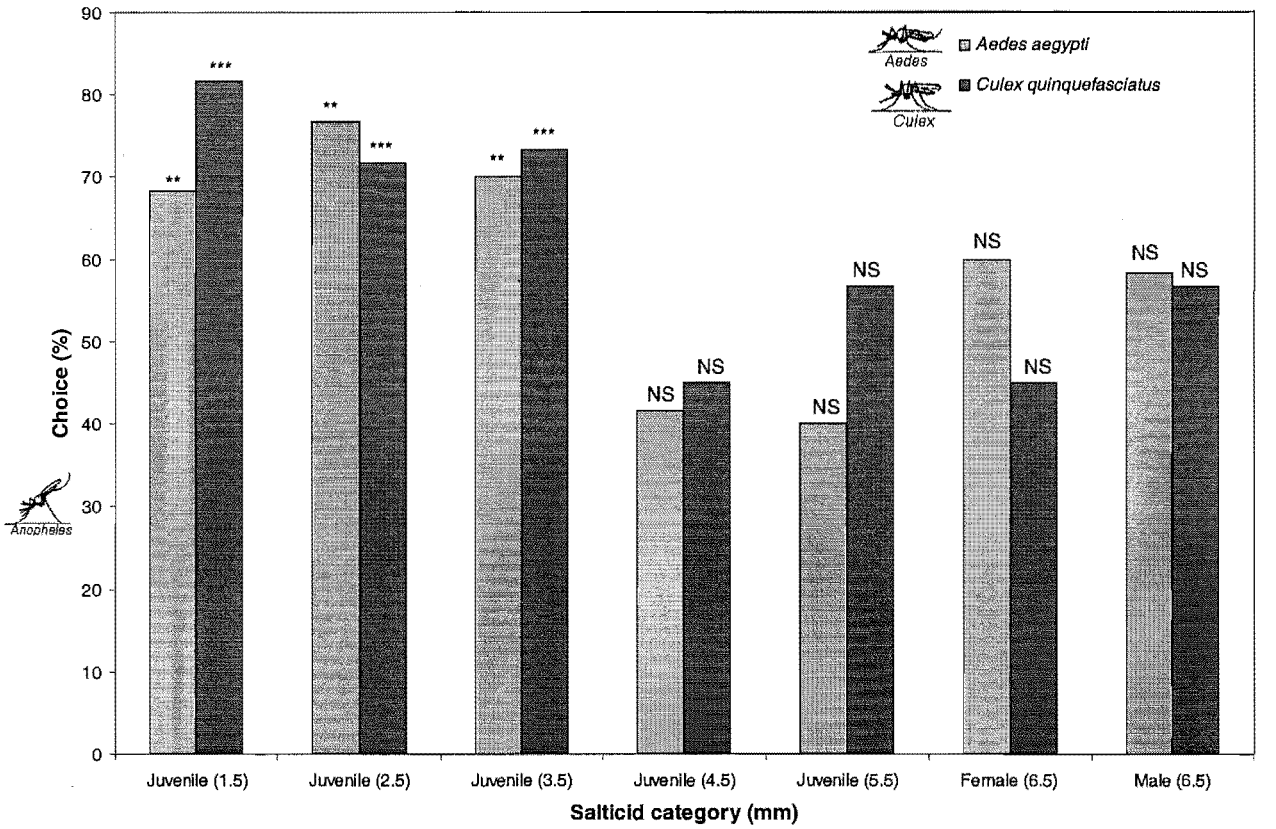

Figure 2. Percentage of times juveniles and adults (body length and sex variable) of *Evarcha culicivora* chose blood-fed female mosquitoes (*Anopheles gambiæ*, body length 4.5 and 5.5 mm) rather than other blood-fed mosquitoes (*Aedes aegypti,* 5.0 mm or blood-fed female *Culex quinquefasciatus*, 4.5 mm) matched for size. N=60 for each bar. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ***P<0.001, **P<0.01). Note characteristic resting posture of *Anopheles* (adapted from http://www.vnh.org/Malaria/app2.html; accessed 31/08/04).

Discussion

Prey size influences *E. culicivora's* prey-choice decisions (Chapter 3, 4) but prey size was not a variable in the present chapter. Here I found evidence that, independent of prey-size preference, the small juveniles of *E. culicivora* choose *Anopheles gambiæ* more often than *Aedes aegypti* and *Culex quinquefasciatus*. Results from tests using virtual mosquitoes indicated that the resting posture is an important cue used by small juveniles of *E. culicivora* to identify mosquitoes from the genus *Anopheles*. This is an important finding because the mosquitoes that serve as vectors for human malaria all belong to this genus (Gillet, 1971). The results from this study raise important questions about *E. culicivora's* role in integrated vector management (IVM) programmes for the control of malaria.

Despite the initial success of drugs such as chloroquine and quinine to treat malaria, drug resistance in the parasite (*Plasmodium*) is now widespread (Hastings *et al.*, 2002a,b; Wellems, 2002). More recently, artemisin (or qinghaosu), a plant-derived terpenoid (Cowan, 1999), has come into wide usage, but the expense of this drug limits its use in Africa and there are arguments for restricting its use to guard against resistance evolving in *Plasmodium*. We now know that insecticides favour the evolution of resistance in insects and antimalarial drugs favour resistance in the parasite, but, with the simultaneous recent publication of the genomes of *Plasmodium falciparum* (Gardener *et al.*, 2002) and its principal vector, *Anopheles gambiæ* (Holt *et al.*, 2002), new avenues are being opened for developing genetically modified *Anopheles* (preventing the transmission of *Plasmodium*), for developing vaccines, and for developing anti-malarial drugs (Kissinger *et al.*, 2002). Although efforts to develop a vaccine for malaria and efforts to replace wild *Anopheles* with transgenic mosquitoes have received a lot of publicity, it is widely acknowledged that neither can be expected to have much impact for well over a decade (Coates, 2000; Alphey *et al.*, 2002; Ito *et al.*, 2002; Miller & Greenwood, 2002; Richie & Saul, 2002; Scott *et al.*, 2002). While vaccination against viral and bacterial diseases has met with considerable success, vaccines against a eukaryotic organism have so far met with dismal failure (Ewald, 2000; Shiff, 2002). Furthermore, the notion that genetic modification will be a magic bullet that solves the malaria problem is dubious. There are strong arguments for a more integrative approach including, as part of the package, the development of combinations of vaccines, effective antimalarial drugs and vector control (Miller & Greenwood, 2002; Shiff, 2002). With over a million people dying from this disease each year (Breman, 2001), most of them in Sub-Saharan Africa (Greenwood & Mutabingwa, 2002), malaria is a health problem needing urgent attention.

In the foreseeable future, drugs continue to be a major tool for the control and treatment of malaria. Nevertheless, there seems to be a real need for field studies of vector ecology (Curtis, 2000; Enserink, 2002; Shiff, 2002) as this may provide avenues for IVM programmes. Although it is widely accepted that the only way to control malaria in Africa is by interrupting the transmission of *Plasmodium* (Miller & Greenwood, 2002), controversy persists over whether it is more effective to target mosquitoes as adults or as larvae.

Remarkably little work has been done on the biocontrol of malaria vectors. The primary exceptions have been attempts to use bacteria and larvivorous fish, both of which target mosquito larvae instead of adults, and both of which have yielded mixed results (see Walker, 2002), largely because of the transient nature of pools in which mosquitoes often lay their eggs (Gimnig *et al.*, 2001). Another drawback of these efforts is that species used for vector control may need to be brought into areas in which they are not endemic, and this may disrupt the ecological balance of endemic species.

Mosquitoes have natural predators living sympatrically with them in all parts of the world. Spiders are recognised as major generalist predators of insects the world over (Bristow, 1941; Wise, 1993). Spiders have also been shown to be predators of the larvae of *Culex pipiens* (Breene *et al.*, 1988), the vector for filariasis, West Nile fever and other diseases (Clements, 1999). However, spiders may play an especially important role as control agents for adult mosquitoes. Strickman *et al.* (1997) suggested that *Crossopriza lyoni* (Pholcidae) may be especially useful in Thailand as a biocontrol agent for *Aedes aegypti*, the vector for yellow fever and dengue (even the small juveniles of *C. lyoni* can catch *Aedes*). However, despite their seeming potential, spiders seem rarely to have been considered as potential agents of biological control of mosquitoes.

Evarcha culicivora is a unique spider, native to the Lake Victoria region of Kenya and Uganda (Weslowoska & Jackson, 2003), that has a specific prey-preference for blood-fed female mosquitoes. Small juveniles of *E. culicivora*, in addition, target *Anopheles* mosquitoes as their preferred prey. This is the first instance in which a preference for *Anopheles*, the vectors of human malaria, has been reported for any animal. Given the desperate situation faced by African people under the 'malaria burden' (Breman, 2001), further studies into the potential use of *E. culicivora* as a biocontrol agent for malaria are urgently needed.

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CHAPTER SIX

Prey-specific predatory tactic of the small juveniles of Evarcha culicivora, *a mosquito-eating jumping spider*

Abstract

The prey-capture behaviour of *Evarcha culicivora*, an East African mosquito-eating jumping spider, was investigated in the laboratory using living prey and using dead, motionless lures made from mosquitoes of two species. In each test, *E. culicivora* was presented with two lures, each mounted in a different life-like posture. Small juveniles of *E. culicivora*, which are known to prefer mosquitoes from a particular genus, *Anopheles*, were more effective at capturing *Anopheles* than *Culex*. Small juveniles that had never encountered mosquitoes from any genus (having been reared on chaoborid and chironomid midges) used a specific predatory tactic to capture *Anopheles*. Posture appeared to be the primary cue by which small juveniles of *E. culicivora* chose predatory tactics. Lures made from *Anopheles* mounted in the *Anopheles* posture and *Culex* mounted in the *Anopheles* posture elicited detour behaviour by small, but not by large, juveniles of *E. culicivora*. However, neither the small nor the large juveniles of *E. culicivora* tended to make detours when approaching lures made from *Anopheles* or *Culex* that were mounted in the *Culex* posture.

Introduction

Pronounced prey-choice behaviour and prey-specific capture behaviour have evolved especially in two groups of jumping spiders (Salticidae), the araneophagic species (i.e., species that prey especially on other spiders) and the myrmecophagic species (i.e., species that prey especially on ants), with the target of this focusing of tactics being sometimes remarkably specific. For example, *Portia fimbriata* from Queensland (Australia) adopts tactics that are specific to a particular prey species, *Euryattus* sp., a common salticid in the same habitat (Jackson & Wilcox, 1990, 1993a). *Euryattus* females are unique among salticids because they make a nest by suspending a dead rolled-up leaf by silk lines from the vegetation. *P. fimbriata* captures *Euryattus* females by mimicking the vibratory courtship displays of *Euryattus* males, luring females out of their nests. Another especially striking example of specificity is seen when the *Portia labiata* from Los Baños in the Philippines stalks *Scytodes pallida*. Being an araneophagic spitting spider that specialises at preying on salticids (Jackson *et al.*, 1998), *S. pallida* is a particularly dangerous prey for *P. labiata*. The tactic *P. labiata*

uses to capture *S. pallida* includes approaching from the scytodid's rear, away from the spitting spider's line-of-fire.

Another example of remarkably specific preference has recently been documented for *Evarcha culicivora*. This is a salticid that feeds indirectly on vertebrate blood by selecting as prey female mosquitoes that are engorged with blood from blood meals. Smaller juveniles of *E. culicivora* have, in addition, a more fine-tuned preference. When their choices are between blood-fed mosquitoes belonging to different genera, they select *Anopheles* instead of *Aedes* or *Culex* (Chapter 5). There is no evidence that adults or the larger juveniles of *E. culicivora* base prey-choice decisions on the genus to which a mosquito belongs. In this chapter, the prey-capture behaviour of *E. culicivora* is documented for the first time based on observing many hundreds of encounters of *E. culicivora* of different sizes with living prey. Then a particular hypothesis is investigated experimentally: that smaller juveniles of *E. culicivora*, but not the larger juveniles, adopt innate *Anopheles*-specific prey-capture behaviour, with the posture of the mosquitoes being an important cue eliciting this behaviour.

Materials and Methods

Experiments using lures

The study was conducted at the International Centre of Insect Physiology and Ecology (ICIPE) Mbita Point Field Station (MPFS) in south-western Kenya and at the University of Canterbury in New Zealand. All living spiders came from laboratory culture, and standard spider-laboratory procedures were adopted (Jackson & Hallas, 1986). All testing was carried out between 0700 h and 1900 h (laboratory photoperiod 12L:12D, lights on at 0700).

Tests were carried out using single lures made from blood-fed female mosquitoes (for details about diet and rearing see: Chapter 3). Two species were used: *Culex quinquefasciatus* and *Anopheles gambiæ* (body length of both species 4.5 mm). Methods for making lures were the same as those used in Chapter 3 and only details specific to these tests will be mentioned.

Lures were made so that they varied in their resting posture. The resting posture of *Anopheles* is with hind legs raised and the abdomen angled up at about a 45° angle from the surface on which the mosquito is standing. When in this posture, the abdomen of *Anopheles* forms a straight line with the proboscis. This characteristic posture readily distinguishes mosquitoes that belong to the genus *Anopheles* from mosquitoes that belong to the genera *Aedes* and *Culex* (Fig. 1).

Mosquitoes from these genera rest with their abdomens held parallel to the substrate and their heads tilted toward the substrate.

Figure 1. Resting posture of *Anopheles*, *Aedes* and *Culex* mosquitoes. Note: *Anopheles* holds posterior body tilted up from substrate and hind legs are raised. Adapted from http://www.vnh.org/Malaria/app2.html (accessed 31/08/04).

Dead specimens were manipulated so that lures made from *Culex* were either in the resting posture typical of *Culex*, or in the resting posture typical of *Anopheles*. Lures made from *Anopheles* were also manipulated so that they were in the resting posture typical of *Culex* or in the resting posture typical of *Anopheles*.

Tests were carried out using 1.5 mm and 3.5 mm (body length) juveniles of *E. culicivora*. Except for 1.5 mm juveniles, all test spiders had been starved for 7 days before testing and no spiders had ever encountered mosquitoes (1.5 mm juveniles had emerged from their brood sacs 5 days before testing and had never encountered prey of any type). No individual of *E. culicivora* and no individual lure was used in more than one test. All individuals of *E. culicivora* that were tested were reared on a diet of chaoborid and chironomid midges only (i.e., they had no prior experience with mosquitoes of any kind).

Apparatus and testing procedures were similar to those detailed elsewhere (Harland & Jackson, 2000) with modifications being made for testing small juvenile spiders. The apparatus was a wooden ramp (40 mm wide and 140 mm long) that, with the support of a wooden dowel (15 mm thick), angled up at 20°. The ramp and supporting dowel were on a wooden base (50 mm wide x 150 mm long x 15 mm thick). A lure was positioned at the top of the ramp, in front a wall which served as a background against which spiders could see the lure. The wall was a 55 mm high and 40 mm wide (15 mm thick) piece of brown wood glued perpendicular to the top end of the ramp. The lure was centred on the ramp *c.* 15 mm from the base of the wall (this left a space of *c.* 10 mm between the wall and the top edge of the cork disc) and angled 45° from forward (i.e., for *E. culicivora*

walking directly up the ramp, the lure would be facing 45° away). For each lure, whether it was facing 45° to the left or 45° to the right was decided at random.

Before testing began, the salticid was kept until quiescent in a covered pit (diameter 30 mm, depth 10mm) drilled into the top surface of the ramp. The starting pit was centred 50 mm from the bottom of the ramp (i.e., the lure was positioned *c.* 40 mm from the top-end of the pit). Tests were allowed to start by removing the cover. After uncovering the pit, tests were aborted if the salticid failed to come out within 30 min or came out, but then moved off the ramp without first moving toward the lure. In successful tests, the salticid moved toward the lure (i.e., walked up the ramp) and contacted the cork disc, the lure, or both. Testing ended when the salticid contacted the lure, when, after contacting the cork disc, it moved off the cork disc, or, after 30 min, if the salticid contacted the cork disc and then stayed there without contacting the lure.

Horizontal orientation of the test spider when approaching the lure was defined as follows: front (no more than 45° to the left or the right of the anterior end of the saggital plane of the lure's head); rear (no more than 45° to the left or the right of the posterior end of the saggital plane of the lure's abdomen); side (between in front and from behind). Approaches to the lure were considered as 'detours' when the salticids approached the lure from the rear, or when salticids approached the lure from the side in the first instance and then moved around to the rear. "Did not detour" was recorded when spiders that approached the lure from the front or approached from the side without shifting to the rear.

Results were analysed using Fisher exact tests and chi-square tests independence using Bonferroni adjustments when multiple comparisons were made (Sokal & Rohlf, 1995).

Prey-capture success

Capture success was determined by testing 1.5 mm and 3.5 mm juveniles of *E. culicivora* with mosquitoes (*Anopheles gambiæ* and *Culex quinquefasciatus*) and lake flies (*Nilodorum brevibucca*). The mosquitoes were, for both species, blood-fed females, sugar-fed females and males. *An. gambiæ* (sensu stricto) mosquitoes were laboratory-reared from a colony established from wild gravid females collected at Mbita Point (Western Kenya) in February 2000. *C. quinquefasciatus* were collected as larvae and reared to adulthood and the chironomid midge, *Nilodorum brevibucca*, was collected from the field as needed.

All observed 1.5 mm juveniles had recently emerged (about 5 days before testing) from their brood sac and had never been exposed to prey of any type (i.e., spiders were unfed). Larger juveniles (3.5 mm) had had a short fast-period of 3-5 days before being tested.

In each test, one juvenile of *E. culicivora* and two live prey (one each of two different types) were put together inside a clear Perspex box (30 cm X 30 cm X 30 cm). Within each species, prey-capture success was not significantly different between blood-fed females, sugar-fed females and males so these groups were pooled. Results were analysed using chi square tests of independence (Sokal & Rohlf, 1995).

Qualitative account of the prey-capture behaviour of *Evarcha culicivora*

Methods

General observations were made on the predatory behaviour of small juveniles (1.5 mm and 3.5 mm), large juveniles (4.5 mm or larger) and adults (6.5 mm) of *E. culicivora*. Prey used for these observations were the same as those used in the tests for prey-capture success.

As in earlier studies (see Jackson & van Olphen, 1991), I use the expressions 'usually' or 'generally', 'sometimes' or 'occasionally', and 'infrequently' or 'rarely' to indicate frequencies of occurrence of 80% or more, 20-80% and 20% or less, respectively.

Behaviour of large juveniles and adults

Large juveniles (4.5 mm) and adults (6.5 mm) of *E. culicivora* often oriented toward their potential prey from a distance of 100 mm or further. Once the spider had oriented toward the prey, it became quiescent, often slowly waving its palps (about one cycle every one or two seconds), before approaching, usually by slowly walking in a direct line (usually from the side of the prey) toward the prey, with occasional short (*c.* 1 s) pauses, slowing down as it got closer. Spiders never ran or jumped to approach prey. When 20-40 mm from the prey, the spider stopped and adopted a distinctive pre-leap posture (legs II-IV highly flexed and pulled in close to the body; body lowered, appearing to touch the substrate; legs I slightly flexed in front, with tarsi angled slightly toward each other).

In the pre-leap posture, the body was kept aligned with the cephalothorax and the palps were stationary and retracted (i.e., close to the face). Before leaping, the spider occasionally flexed and pulled its legs I close to the body and remained completely still in this posture (either with legs I close to the body or in front of the body), until finally leaping on the prey. When in this position, the

spider sometimes moved toward the prey by using legs I, II and III to almost 'drag' the body along the ground, a millimetre or less at a time, while keeping legs close to the body. These approaches could take as little as a few seconds or as long as 2 or 3 min. Having leapt on their prey, spiders usually landed on the dorsal surface of the prey.

Behaviour of small juveniles

Like large juveniles and adults, small juveniles of *E. culicivora* (body length, 1.5-3.5 mm) became quiescent when they oriented toward their potential prey. If *c.* 50 mm or further away when it oriented toward its prey, the spider usually approached by running or jumping in short bursts toward the potential prey. The distance covered in each burst of speed was about 10 mm. The pauses between bursts varied from a few seconds to about 2 min. Between bursts, the spider stopped for a variable amount of time while keeping its gaze fixed on the prey and then ran or jumped towards it again, repeating this sequence until 10-15 mm away from the prey.

If the potential prey was close (< 15 mm) to the spider when the spider oriented toward it, *E. culicivora* usually approached by walking very slowly towards the prey in a manner similar to that described for adults and larger juveniles when in their pre-leap posture. As with adults and large juveniles of *E. culicivora*, small juveniles approached prey in bursts (i.e., in intermittent bouts of rapid movement), but occasionally spiders switched from running or jumping to slowly walking, until reaching a distance of 10-15 mm from the prey.

When the spider got within 10-15 mm of its prey, other than *Anopheles*, it usually continued its approach in a direct line, either by walking slowly (and slowing down as it got closer to the prey) or by jumping until very close (2-3 mm from the prey) and then resuming slow walking until it finally attacked the prey. If the prey was *Anopheles* and the spider was facing the antero-lateral end of the mosquito, the spider circled around the mosquito (sometimes moving slightly away from it to do so) until it faced the postero-lateral end of the mosquito (i.e., it made a 'detour'). Detours usually occurred only when the prey was *Anopheles*. Once oriented toward the postero-lateral end of the mosquito, *E. culicivora* walked between the *Anopheles'* legs and, when directly underneath its abdomen, leapt up to bite it (i.e., to grab hold with its chelicerae), usually on the posterior ventral thorax (Fig. 2). When attacked, the mosquito generally started flying, but the spider held on, with the flying mosquito falling to the ground 15-60 s later. Attempts by small juveniles to capture *Anopheles* were usually successful.

Figure 2. Juveniles of *Evarcha culicivora* (*c.* 3.5 mm) feeding from the ventral thorax of blood-fed females of *Anopheles gambiæ*. *E. culicivora* has moved around the mosquito after capture (if not it would be facing the mosquito's head, because they usually grab hold of the posterior ventral thorax of the mosquito from underneath). Note size of predator relative to the size of its prey. Photos courtesy of Robert Jackson.

If *E. culicivora's* prey was something other than *Anopheles*, spiders did not attempt to get underneath the prey and instead jumped on it from as far away as 3-4 mm, usually biting the prey on its thorax. If the prey was *Culex*, attempts by small juveniles to capture the prey were only occasionally successful.

Results

Experiments using lures

When the lures were made from *Anopheles*, significantly more 1.5 mm juveniles (χ^2=43.46, P<0.001, N=100), but not 3.5 mm juveniles (χ^2=0.04, NS, N=90) of *E. culicivora* detoured when stalking lures of *Anopheles* resting in an *Anopheles* position than in a *Culex* position. When the lures were made from *Culex*, significantly more 1.5 mm juveniles (χ^2=20.40, P<0.001, N=80), and 3.5 mm juveniles (P<0.05, N=90) of *E. culicivora* detoured when stalking lures resting in an *Anopheles* position than in a *Culex* position (Fig. 3).

Significantly more 1.5 mm juveniles used detours to approach *Anopheles* resting in an *Anopheles* position than 3.5 mm juveniles (χ^2=61.45, P<0.001, N=100) and significantly more 1.5

mm juveniles used detours to approach *Culex* resting in an *Anopheles* position than 3.5 mm juveniles (χ^2=44.98, P<0.001, N=90). There was no significant difference in the number of 1.5 mm juveniles using detours to approach *Anopheles* resting in a *Culex* position than the number of 3.5 mm juveniles using detours to approach *Anopheles* resting in a *Culex* position (χ^2=1.88, NS, N=90). However, significantly more 1.5 mm juveniles used detours to approach *Culex* resting in a *Culex* position than 3.5 mm juveniles (P<0.001, N=80) (Fig. 3).

Prey-capture success

Both 1.5 mm (χ^2=12.00, P<0.001, N=36) and 3.5 mm (χ^2=31.16, P<0.001, N=151) juveniles of *E. culicivora* were significantly more successful at catching *Anopheles gambiæ* than *Culex quinquefasciatus* (Fig. 4). The 3.5 mm juveniles were significantly more successful at capturing *Nilodorum brevibucca* than *C. quinquefacsitaus* (χ^2=6.24, P<0.05, N=61) but there was no significant difference in the capture success of 3.5 mm juveniles when the two prey were *N. brevibucca* and *An. gambiæ* (χ^2=3.45, NS, N=154) (Fig. 4). No 1.5 mm juveniles were observed attacking *N. brevibucca.*

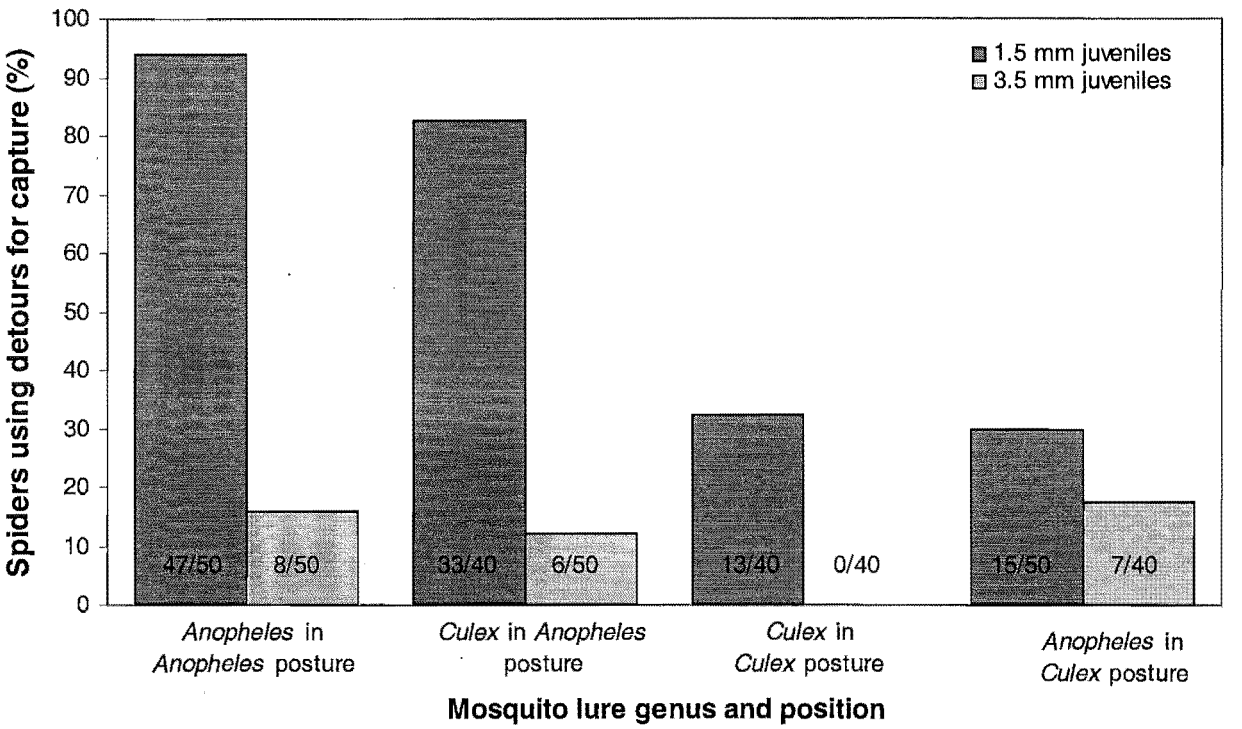

Figure 3. Percentage of juveniles of *Evarcha culicivora* (1.5 mm and 3.5 mm body length) that stalked lures made from dead mosquitoes in different resting postures. Test spider had access to one type of prey (*Anopheles gambiæ* in *Anopheles* resting posture, *An. gambiæ* in *Culex* resting posture, *Culex quinquefasciatus* in *Anopheles* resting posture, *C. quinquefasciatus* in *Culex* resting posture). Number of test spiders that went toward each lure using a detour divided by sample size indicated in each bar.

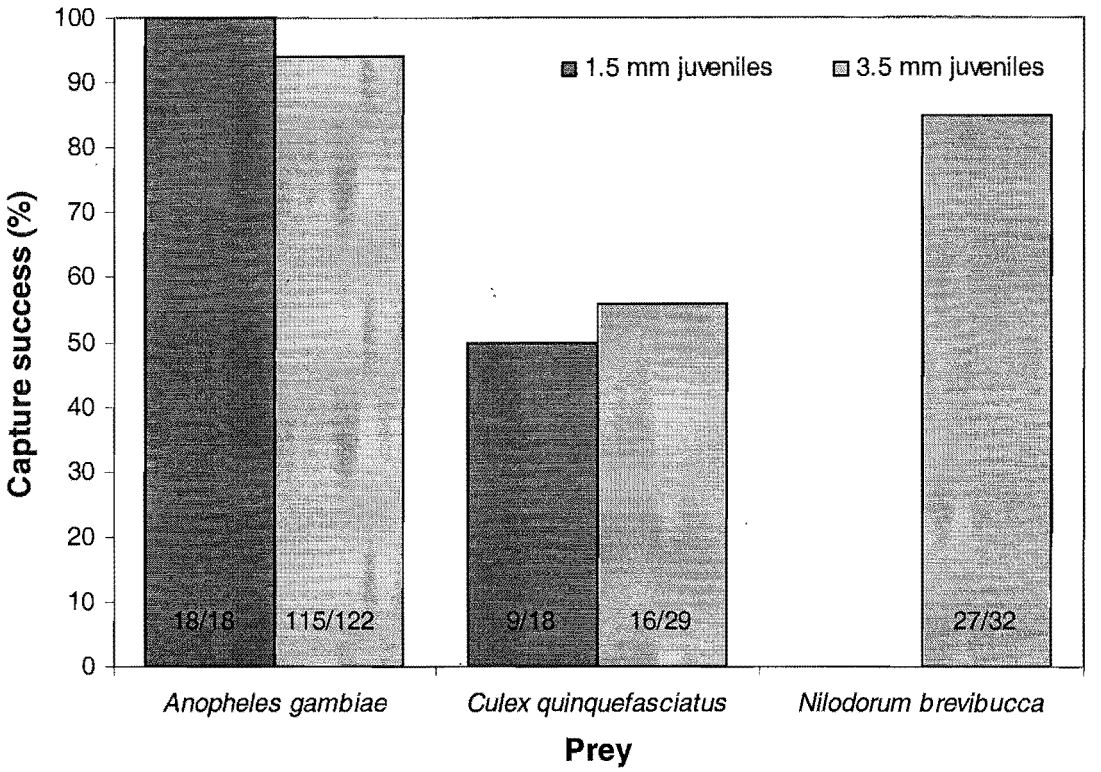

Figure 4. Live prey capture success by juveniles of *Evarcha culicivora* (1.5 mm and 3.5 mm body length) (*Anopheles gambiæ* & *Culex quinquefasciatus*: mosquitoes; *Nilodorum brevibucca*: midge). Number of test spiders that caught each prey type divided by sample size indicated in each bar.

Discussion

The findings from this chapter, and from chapters 3 and 5, suggest that the predatory strategy of the small juveniles of *E. culicivora* has become adaptively fine-tuned to *Anopheles* as prey. The earlier studies (Chapters 3 & 5) showed that juveniles of *E. culicivora* have a pronounced preference for *Anopheles* in prey-choice tests, and the findings in this chapter suggest that they are considerably more effective at capturing *Anopheles* than at capturing *Culex*. Evidently, small juveniles also have an innate *Anopheles*-specific prey-capture tactic, and the use of this tactic probably helps account for the small juvenile's effectiveness at capturing *Anopheles*. A primary cue for the *Anopheles*-specific tactic appears to be the mosquito's posture.

The resting posture of *Anopheles* is distinctive (Fig. 1): body tilted 45° to the substrate, proboscis touching the substrate and the posterior tip of the abdomen in the air. *Anopheles* mosquitoes also rest with their hind legs raised in the air, with the first and second pairs spread widely apart and resting on the substrate. By adopting this resting posture, *Anopheles* appears to become vulnerable to exploitation by the tactic adopted by small juveniles. These small spiders can, by sight, identify the mosquito's posture and then move under the mosquito's raised abdomen from behind. Other mosquitoes, by resting with their bodies held parallel to the substrate, appear to be less vulnerable to the small juveniles of *E. culicivora*, probably because it is harder for *E. culicivora* to creep under them.

Other instances of salticids using prey-specific prey-capture behaviour are known (Edwards & Jackson, 1993, 1994; Bartos, 2002), with the most pronounced examples being species that specialise on ants (Jackson & van Olphen, 1991, 1992; Jackson & Wilcox, 1993b; Jackson *et al.*, 1998; Li & Jackson, 1996a; Li *et al.*, 1996; Li *et al.*, 1999; Jackson & Li, 2001) and species that specialise on other spiders (Li & Jackson, 1996b; Li *et al.*, 1997; Jackson & Li, 1998; Jackson, 2000; Harland & Jackson, 2001; Cerveira *et al.*, 2003). Ants and spiders are dangerous prey for a salticid because they have weapons (strong mandibles and chelicerae, venom, and so forth) with which they can seriously (sometimes fatally) harm the salticid that attempts to capture them. *E. culicivora* seems to be an unusual example of a salticid that adopts a distinctive prey-specific capture behaviour because, unlike ants as prey (Gillespie & Reimer, 1993; Vieira & Hoefer, 1994; Halaj *et al.*, 1997; Nelson *et al.*, 2004) and unlike other spiders as prey (Bristowe, 1941; Foelix, 1996; Persons & Rypastra, 2000; Barnes *et al.*, 2002), mosquitoes cannot bite and otherwise inflict injury directly on the salticid predator. For small juveniles of *E. culicivora*, however, a mosquito may be indirectly dangerous. It can fly away after the spider grabs hold of it, with the spider

possibly being physically at risk because of losing control over where it might land (e.g., in water, in a spider web or among ants). A more significant danger might be the risk of losing the prey. *Anopheles'* posture appears to afford small juveniles of *E. culicivora* with the means of getting close to the mosquito without first alerting it and getting a firm grip from underneath before the mosquito attempts to escape.

Evidently, the tactic *E. culicivora* adopts when it sees a mosquito in the *Anopheles* posture is innate (the individuals used in this study had no prior experience with *Anopheles*). This innate prey-capture tactic (taking detours and attacking from behind and underneath the mosquito) is remarkably prey-specific because it is used for capturing specifically *Anopheles*. It is also specific in another way; and this other way appears not to have been demonstrated before in research on salticids. This particular predatory tactic is used by only a specific stage in the life cycle of *E. culicivora* (i.e., it is used only by the small juveniles). At about 3.5 mm long, *E. culicivora* begins to become non-specific in the predatory behaviour it adopts for different mosquito genera. When, at about 3.5 mm, juveniles of *E. culicivora* appear to be large enough not to need the *Anopheles*-specific tactic. They are large enough to approach directly and overpower the mosquito by leaping. Notably, despite their small size, 1.5 mm juveniles appeared to be as successful at capturing prey as larger juveniles, testifying to the highly successful predatory tactic employed by the smaller juveniles.

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CHAPTER SEVEN

Colour vision in Evarcha culicivora

Abstract

The influence of colour cues on the prey-choice decisions of *Evarcha culicivora* was investigated by varying the colour of virtual prey made using computer animation. As *E. culicivora* feeds indirectly on vertebrate blood by choosing as prey female mosquitoes that are carrying blood, *Anopheles* mosquitoes were used as the template for the virtual prey, with the prey having black, blue or red abdomens on otherwise identical bodies. All size categories of *E. culicivora* distinguished red from black, choosing the mosquitoes with red abdomens more often than mosquitoes with black abdomens. Adults and large juveniles of *E. culicivora* distinguished red from blue, choosing the mosquitoes with red abdomens more often than mosquitoes with blue abdomens. However, for small juveniles of *E. culicivora*, neither colour was chosen significantly more often then the other, suggesting that the spider's ability to discern colour improves with increasing eye-size. Findings are discussed in relation to salticid colour vision.

Introduction

Colour vision is generally based on an animal having photopigments with at least two different spectral sensitivities. This allows the animal to discriminate between different wavelength distributions of light. Light of different wavelengths will result in unique ratios of stimulation of the two pigments contained in photoreceptor cells, independent of the total stimulation or level of illumination. For animals with only one photopigment, wavelength and intensity cannot be disentangled from one another and colour vision is impossible (Land, 1981; Land & Nilsson 2002).

For human eyes, the visible spectrum lies between 400 ('violet') and 700 nm ('red'). However, it is important to distinguish between colour and wavelength. Leaves look green (colour) and reflect light most strongly at wavelengths between 500-600 nm, whereas blood looks red (a colour very different from green) and yet reflects light most strongly at wavelengths not much longer than the 'green' of leaves (between 600-650 nm). The discrete jump between the appearance of the colours (and not the reflected wavelength) is caused by our having three types of photoreceptors (cones) that are maximally sensitive to light in the green, red and blue regions. However, we see more than these three colours. This is because colour is the subjective result of

wavelength analysis. The cones in our eyes are stimulated differentially depending on the reflected wavelength we are looking at (e.g., the reflected wavelength from slate). We do not have 'grey' cones; the grey appearance of slate is, instead, a summation of the relative intensities of stimulation in each of the three cones types that we *do* have. In other words, most colours are essentially an equation in our heads. Confusion arises because, although the colour we see depends on the relative stimulation of our three cone types, we also refer to certain spectral wavelengths by using colour names (e.g., we refer to 580 nm as yellow). In fact, 'yellow' may just be the correct mixture of 620 nm 'red' and 540 nm 'green' wavelengths, rather than 'pure' 580 nm reflected light. In humans, colour is also a product of processing at a level higher than the receptors in the eye. Consequently, the question of how other animals experience colour appears to be intractable. When referring to colours in this chapter, I am using names of colours referring to specific wavelengths of light and do not imply that the 'red' perceived by the test spiders is the same as the 'red' I perceive.

There is considerable evidence that the anterior median (AM) eyes of salticids can support colour vision (Land, 1969a,b; De Voe, 1975, Yamashita & Tateda, 1976; Blest *et al.*, 1981; Blest *et al.*, 1990), despite little being known about how colour actually influences salticid behaviour. For example, many salticids are brightly coloured, and the intraspecific displays of these species appear to show-off their colouration.

Getting experimental evidence of the role of colour in salticid behaviour is hindered by the inherent difficulties of manipulating colour on real animals, and attempts to determine the spectral sensitivities of the AM eyes of salticids have yielded mixed results. Perhaps the only study that has directly addressed colour vision in salticids used an ingenious method of heating differently coloured papers and relying on the salticid's (*Hasarius adansoni*) learnt association between colour and heat when avoiding heat (Nakamura & Yamashita, 2000). These authors concluded that *H. adansoni* could detect green, yellow, red and blue.

Evidence suggests variously that salticids have dichromatic (De Voe, 1975; Blest *et al.*, 1981), trichromatic (Land, 1969a), and even tetrachromatic vision (Yamashita & Tateda, 1976). Some studies have suggested that salticids can detect long-wavelength light (red) (Land, 1969a; Peaslee & Wilson, 1989), but others have suggested they cannot (De Voe, 1975; Yamashita & Tateda, 1976; Blest *et al.*, 1981).

Variation in the spectral sensitivities of salticid eyes might be niche-driven, with different salticids having evolved spectral sensitivities adapted to the local ecological pressures. My hypothesis is that the ability to detect red is especially important for the East African salticid

Evarcha culicivora. This salticid feeds indirectly on vertebrate blood by choosing as prey blood-fed female mosquitoes. It can identify its preferred prey by using visual cues alone (Chapter 3). Mosquitoes that have recently fed on blood become engorged and their abdomens typically have a distinctive red colour. The shape of the mosquito is a visual cue to which *E. culicivora* attends (Chapter 4). Here I consider whether colour is also a cue used by *E. culicivora* to identify blood-filled mosquitoes.

In this chapter I explore whether *E. culicivora* distinguishes red from other colours by using virtual mosquitoes that differed in the colour of their abdomens. Specifically, I examine the responses of *E. culicivora* from several size categories to computer-generated virtual prey based on *Anopheles* mosquitoes. These virtual prey differed in that they either had a black, a blue or a red abdomen.

Whether *E. culicivora* detects red was investigated by presenting the salticid with a choice of two mosquitoes, one with a red abdomen and one with a black abdomen. The rationale was a prediction that if *E. culicivora* cannot detect red, it would see red as black (no colour) and choose black prey and red prey equally often. Subsequently, by presenting the salticid with an opportunity to choose between red and blue virtual prey, I tested specifically whether *E. culicivora* can distinguish between short and long wavelength light.

Materials and Methods

The study was conducted at the Spider Laboratory at the University of Canterbury in Christchurch, New Zealand. All living spiders came from laboratory culture, and standard spider-laboratory procedures were adopted (Jackson & Hallas, 1986).

All spiders were measured accurately to the nearest 0.5 mm and placed in categories separated by increments of 1 mm so that each individual fell unambiguously into only one particular size category. Tests were carried out using adult male and female *E. culicivora* (body length of both sexes, 4.5 and 5.5 mm) as well as juveniles of different size classes (body length, 1.5, 2.5 and 3.5 mm). Results from testing male and female *E. culicivora* were never statistically distinguishable and consequently were pooled. All spiders used in tests had been subjected to a short pre-test fast of 5-7 days.

Spiders were presented with virtual mosquitoes (for drawing and animation methods, see Appendix I) Projection methods (Fig. 1) were the same as described in Chapter 4 and only details

specific to this study will be referred to here. Testing methods (criteria for beginning tests and for successful tests) were also identical to those described in detail in Chapter 4.

Virtual mosquitoes were projected side-on so that cues from the head as well as cues from the abdomen were visible to spiders. All virtual mosquitoes measured 3.2 mm (body length) on the glass screen. Virtual mosquitoes were derived from blood-fed females of *Anopheles* and were animated to be grooming at short intervals (for details, see Appendix I). Choice tests were carried out using two virtual mosquitoes, placed side by side, which differed only in the colouration of their engorged abdomens. In the first test, one mosquito had a black abdomen and the other mosquito had a red abdomen. In the second test, one mosquito had a blue abdomen while the other had a red abdomen. Which virtual mosquito was on the left-hand side of the screen or on the right-hand side of the screen was randomised (animations were made for both sides of the screen).

Preliminary analysis of the reflected wavelength of the red in the virtual mosquito with a red abdomen was made using a manual spectrometer made by TTR Optics (Waltham, Massachusetts).

Results of prey-choice tests were analysed using chi-square tests for goodness of fit (null hypothesis: the two choices are made equally often), Fisher exact tests and chi-square tests of independence, using Bonferroni adjustments when multiple comparisons were made (Sokal & Rohlf, 1995).

Results

Preliminary results from spectrometry of the red colour in the mosquito with the red abdomen suggested that the reflected wavelength was *c.* 600 nm. In tests in which one virtual *Anopheles* had a black abdomen and the other virtual *Anopheles* had a red abdomen, juveniles from all size categories (1.5, 2.5 and 3.5 mm) and adults (4.5 and 5.5 mm) of *E. culicivora* chose the mosquito with the red abdomen significantly more often than the mosquito with the black abdomen (Fig. 2).

When one virtual *Anopheles* had a blue abdomen and the other virtual *Anopheles* had a red abdomen, 3.5 mm juveniles and adults (4.5-5.5 mm) of *E. culicivora* chose the mosquito with the red abdomen significantly more often than the mosquito with the black abdomen (Fig. 3). However, there was no significant difference in the treatment of the lures by 1.5 mm juveniles of *E. culicivora* (Fig. 3).

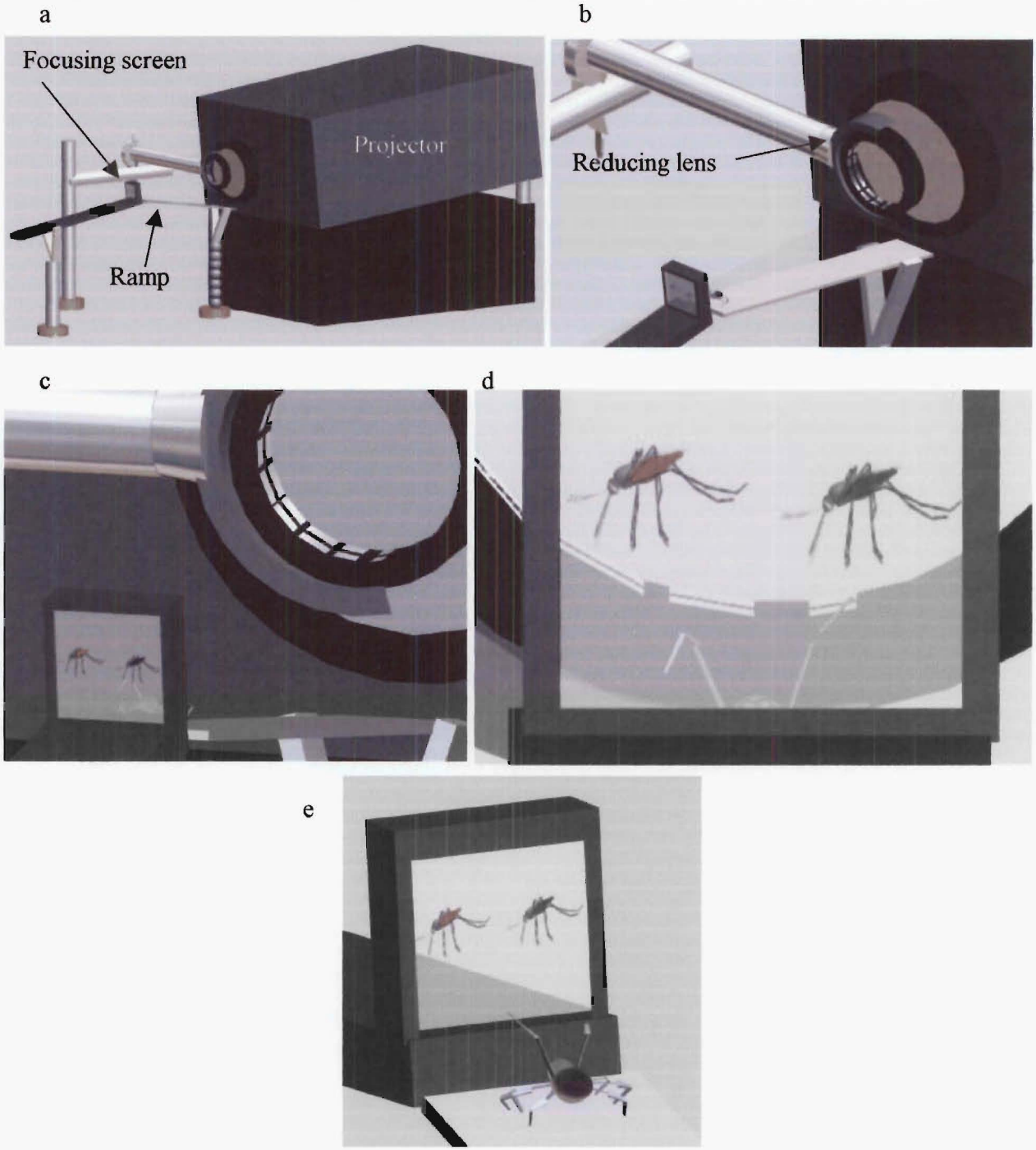

Figure 1. Animation methods. **a)** Experimental set-up (magnetic steel platform on which ramps were placed not shown). **b)** Close-up view of spider on ramp observing virtual prey projected through reducing lens onto focusing screen. **c)** Enlarged view of spider observing virtual prey with red and with black abdomen (reducing screen seen in background). **d)** Spider seen from behind the focusing screen watching virtual prey. **e)** Spider seen from in front of the focusing screen watching virtual prey. Note: spider not to scale.

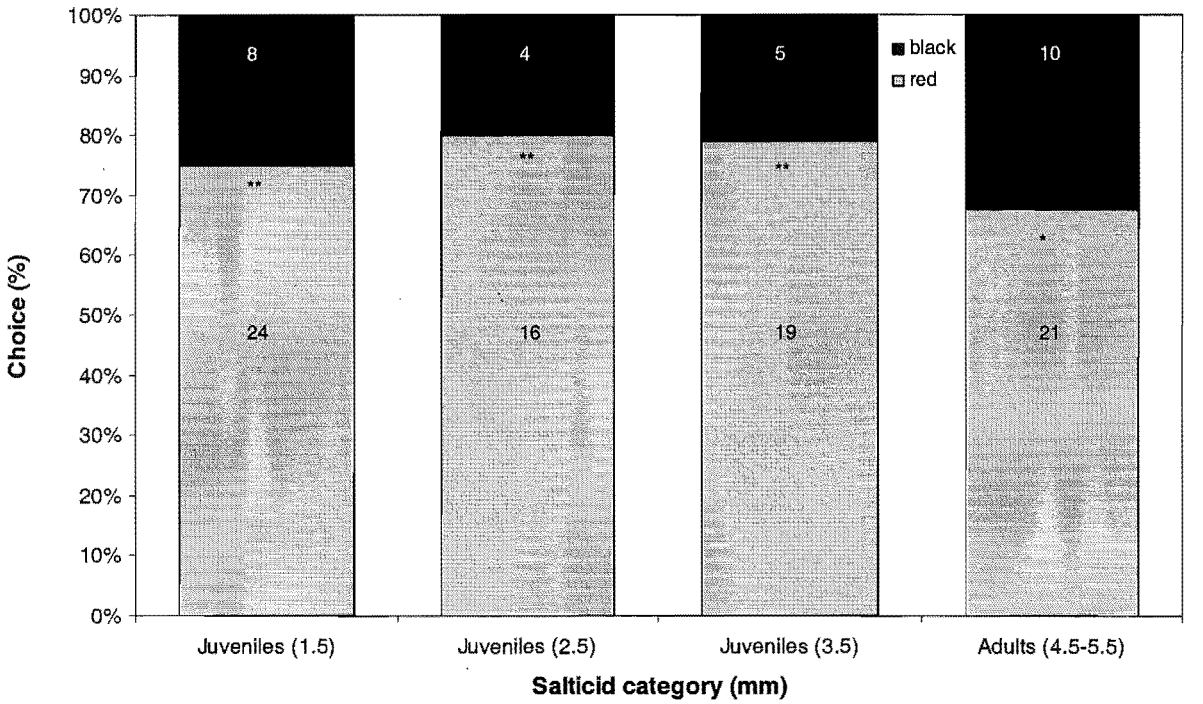

Figure 2. Percentage of times juveniles and adults (body length variable) of *Evarcha culicivora* chose differently coloured virtual lures. Test spider had simultaneous access to two virtual lures based on blood-fed females of *Anopheles gambiæ* mosquitoes. All choice tests with identical virtual mosquitoes that differed in the 'colour' of the abdomen. One mosquito: red abdomen. Other mosquito: black abdomen. Virtual mosquitoes same size (3.2 mm). Numbers of spiders that chose each prey shown in bars. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; ** $P<0.01$, * $P<0.05$).

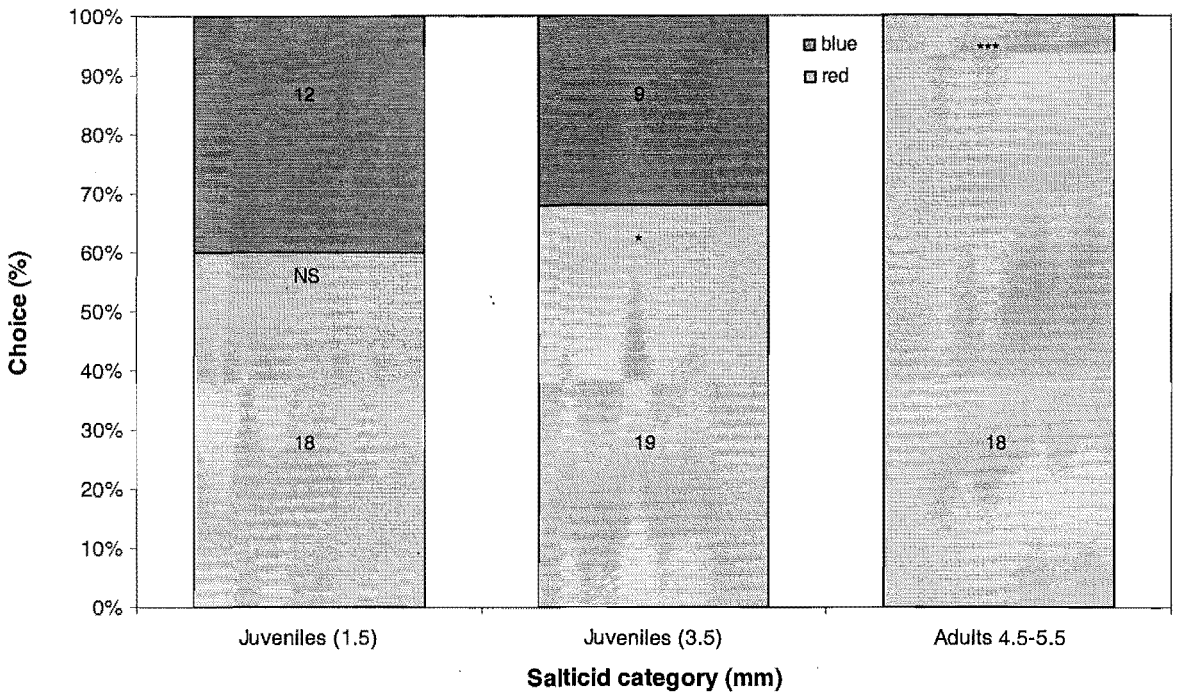

Figure 3. Percentage of times juveniles and adults (body length variable) of *Evarcha culicivora* chose differently coloured virtual lures. Test spider had simultaneous access to two virtual lures based on blood-fed females of *Anopheles gambiæ* mosquitoes. All choice tests with identical virtual mosquitoes that differed in the 'colour' of the abdomen. One mosquito: red abdomen. Other mosquito: blue abdomen. Virtual mosquitoes same size (3.2 mm). Numbers of spiders that chose each prey shown in bars. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often; *** $P<0.001$, * $P<0.05$).

There was no significant difference in the response to lures with black abdomens and with red abdomens by 1.5 mm and 2.5 mm juveniles (P=0.25, NS, N=52), by 1.5 mm and 3.5 mm juveniles (χ^2=0.13, NS, N=56) and by 1.5 mm juveniles and adults (χ^2=0.41, NS, N=63). There was also no significant difference in the responses toward lures with black abdomens and with red abdomens by 2.5 mm and 3.5 mm juveniles (P=0.29, NS, N=44), by 2.5 mm juveniles and adults (P=0.17, NS, N=51), or by 3.5 mm juveniles and adults (χ^2=0.89, NS, N=55).

There was a significant difference in the response to lures with blue abdomens and with red abdomens. The choices made by 1.5 mm spiders and adults were significantly different from each other, with adults choosing the virtual prey with a red abdomen significantly more often than 1.5 mm juveniles (P<0.01, N=48). Adults also chose the virtual prey with the red abdomen significantly more often than 3.5 mm juveniles (P<0.01, N=46), but there was no significant difference between the choices made by 1.5 and 3.5 mm juveniles (χ^2=0.39, NS, N=58).

Discussion

Salticids of all size classes chose virtual mosquitoes with red abdomens more often than virtual mosquitoes with black abdomens. Preliminary recordings of the reflected wavelength of the red indicated that the reflected light from the red abdomen was of *c.* 600 nm in wavelength. If *E. culicivora* had been unable to detect long wavelength light, it would have been expected to choose mosquitoes with red abdomens and with black abdomens about equally often.

E. culicivora identifies its preferred prey (blood-filled mosquitoes) on the basis on optical cues (Chapter 3). The engorged shape of the abdomen seems to be especially important (Chapter 4), and perhaps shape is an easily identifiable feature for the spider to discern the presence of blood. In animals that can distinguish red, the other easily identifiable feature that indicates the presence of blood is the red colour of the engorged abdomen of the mosquito. The results in this chapter suggest that *E. culicivora* can also use the colour of the mosquito's abdomen as a cue identifying it as blood-fed.

E. culicivora also appears to distinguish between blue (short wavelength) light and red (long wavelength) light. Comparing the findings from testing different size classes of *E. culicivora* suggests that *E. culicivora's* ability to discriminate between colours improves with increasing eye-size. Although the larger individuals of *E. culicivora* discriminated between the mosquito with the blue abdomen and the mosquito with the red abdomen, and chose to attack the mosquito with the red abdomen more often, small juveniles of *E. culicivora* (body length, 1.5 mm) chose the two colours

of mosquitoes at similar frequencies. These results suggest that the eyes of very young salticids may not be structured for effective colour vision. Perhaps their small size limits their ability to process colour.

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CHAPTER EIGHT

Blood-feeding spider seeks plants: Evarcha culicivora *chooses the odour of* Lantana camara

Abstract

Lantana camara is an abundant introduced weed in Western Kenya, where the mosquito-eating salticid, *Evarcha culicivora*, makes its home. In experiments using a Y-shaped olfactometer, *E. culicivora* was tested with the odour of flowers from *L. camara* and flowers from a native weed, *Striga hermonthica*. *E. culicivora* chose the odour of *L. camara* flowers significantly more often than it chose a control with no odour, but did not choose the odour of *S. hermonthica* flowers more than the control with no odour. When tested in olfactory choice tests between flowers from the two species, *E. culicivora* chose the odour of *L. camara* flowers significantly more often than it chose the odour of *S. hermonthica*.

Introduction

Evarcha culicivora is an East African salticid that has unique prey-choice behaviour. *E. culicivora* chooses blood-fed female mosquitoes as its preferred prey and can identify its preferred prey by using odour alone (Chapter 3). However, *E. culicivora* supplements its blood diet by feeding on other insects, such as chaoborid and chironomid midges (lake flies), and on nectar.

Trophic switching (Cohen, 1996), or omnivory, are common themes in the evolution of predatory insects, with numerous predatory insects being known to feed facultatively on plants and plant products (Smith, 1965; Coll & Ridgway, 1995; Coll, 1996; Coll & Izraylevich, 1997), including nectar and pollen. However, use of plants and plant products as food is not a widely appreciated feature of spider biology. Nevertheless, in a comprehensive study based on observations of more than 30 species of salticids being observed feeding on nectar in the field, Jackson *et al.* (2001) studied nectar-feeding in 90 species of salticids and found that all species tested fed on nectar and chose sucrose solution to water in preference tests in the laboratory. These results suggest that nectar feeding is a widespread, if not routine, feeding supplement for salticids. Although there have been no previous reports of nectar-feeding by a salticid being linked to a particular plant species, *E.*

culicivora in the field appear to be especially often found on two plants, *Lantana camara* and *Ricinus communis* (RRJ, unpublished data).

In this chapter I describe three experiments to determine whether *E. culicivora* can distinguish between flowers from two different plants, *Lantana camara* (Verbenaceae) and *Striga hermonthica* (Scrophulariaceae), using odour cues alone. My hypothesis is that *E. culicivora* is attracted specifically to the odour of *L. camara*.

Ricinus communis and *Striga hermonthica* are native to the region in which *E. culicivora* is found, but *Lantana camara*, wild sage, is Central American in origin (most likely from the West Indies, Schemske, 1983) and elsewhere is considered a weed. *L. camara* is a prolific shrub that can reach 3 m or more in height. *L. camara* have dense clusters of colourful flowers (Fig. 1) that change colour (the colour of flowers is highly variable and may change from yellow to red, pink to purple etc.) with age, although only young flowers secrete nectar (Schemske, 1983). In warm climates such as that found at Mbita Point in Kenya, *L. camara* may flower throughout the year (Schemske, 1983). *Striga hermonthica* (commonly known as witchweed) is a flowering parasitic plant that has had a devastating effect on crop production in the savannah regions of sub-Saharan Africa, to which it is native. *S. hermonthica*, like *L. camara*, has flowers that vary in colour (from yellow to red) (Berner *et al.*, 1997).

Figure 1. Male *Evarcha culicivora* on *Lantana camara*. Photo courtesy of Robert Jackson

Materials and Methods

This study was conducted at the International Centre of Insect Physiology and Ecology (ICIPE) Mbita Point Field Station in south-western Kenya. All living spiders came from laboratory culture, and standard spider-laboratory procedures were adopted (Jackson & Hallas, 1986). All testing was carried out between 0700 h and 1800 h (laboratory photoperiod 12L:12D, lights on at 0700).

Methods for using the olfactometer were identical to those described in Chapter 3 except that instead of being presented with odour from prey through the olfactometer, spiders were presented with odour from plants that were not visible to the spider. The plants used were *L. camara* and *S. hermonthica*.

Individuals of *E. culicivora* were given a choice between the odour of *L. camara* flowers and a control of no odour, between the odour of *S. hermonthica* flowers and a control of no odour or between the odour of *L. camara* flowers and the odour of *S. hermonthica* flowers.

Tests were carried out using adult male and female *E. culicivora* that had been starved for 7 days before testing. No test spider was used more than once. Results from male and female *E. culicivora* were not statistically distinguishable and were pooled.

Results

Evarcha culicivora chose the odour of *L. camara* flowers significantly more often than they chose the control (no odour) (χ^2=14.76, P<0.001, N=83, Fig. 2) but did not chose the odour of *S. hermonthica* significantly more than they chose the control (no odour) (χ^2=0.29, NS, N=54, Fig. 2). Furthermore, when flowers from both plants were tested simultaneously, *E. culicivora* chose the odour of *L. camara* flowers significantly more often than they chose the odour of *S. hermonthica* flowers (χ^2=13.93, P<0.001, N=69, Fig. 2).

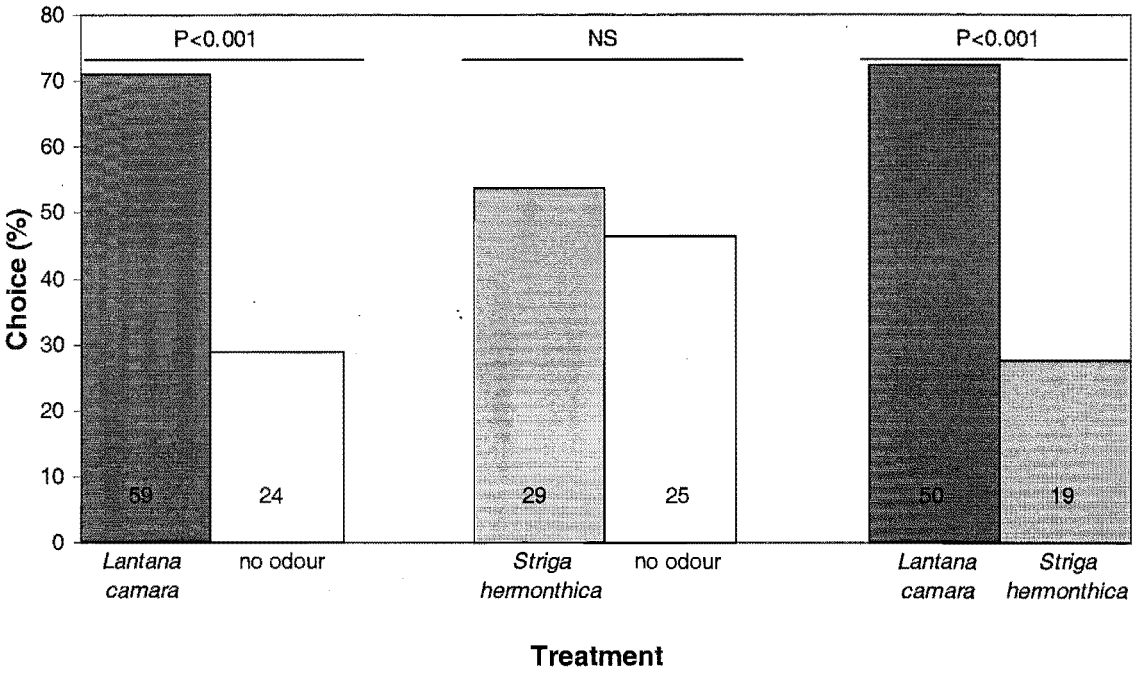

Figure 2. Percentage of adult *Evarcha culicivora* that chose odours from different sources presented simultaneously. First test: spider had access to odour from *Lantana camara* flowers and a control of no odour. Second test: spider had access to odour from *Striga hermonthica* flowers and a control of no odour. Third test: spider had access to odour from *Lantana camara* flowers and odour from *Striga hermonthica* flowers. Numbers that went towards each odour source shown within each bar. Chi-square tests of goodness of fit (null hypothesis: choose each odour equally often).

Discussion

Evarcha culicivora has well-developed chemical senses (Chapter 3). Spiders have been reported to use chemical cues for prey detection, in the discrimination of unpalatable prey types and in predator recognition (Persons & Rypstra, 2000; Persons *et al.*, 2001; 2002; Uetz & Roberts, 2002; Chapter 3), but *E. culicivora* is unusual because, as shown in this study, it can use chemical cues to discriminate between different plants and is attracted to the odour of the plant *Lantana camara* as well as to the odour of *Ricinus communis* (Cross, 2003).

Although *L. camara* is a weed in Africa, there are several native *Lantana* species that may be being displaced by this vigorous species. If the association between *E. culicivora* and *R. communis* (see Cross, 2003) and *L. camara* is a case of co-evolution, it may be that adaptations co-evolved with native *Lantana* are being expressed with the more abundant *L. camara*. Testing the effect of native species of *Lantana* with *E. culicivora* and determining if the nectar from *L. camara*, native *Lantana* and *R. communis* has beneficial effects on *E. culicivora* would help our understanding of how this association may have evolved.

Floral nectaries function to attract pollinators (Gardener & Gillman, 2002). In addition to floral nectaries, some plants, such as *Ricinus communis*, have extrafloral nectaries (EFNs). EFNs can occur in many locations on a plant. For example, in *R. communis*, they are found at the leaf base, on the upper side of the petiole and on the stems, close to the leaf attachments (van Rijn & Tanigoshi, 1999). Secretion of nectar from an EFN may serve to attract predaceous arthropods, such as ants (Oliveira, 1997), mites (Pemberton, 1993), ladybirds (Pemberton & Vandenberg, 1993) and spiders (Ruhren & Handel, 1999). There is evidence that the EFNs of damaged *R. communis* secrete more nectar than those of undamaged plants (Wäckers *et al.*, 2001). In addition, plant compounds affect the fecundity of predatory mites (Dabrowski & Bielak, 1978) and extra-floral nectar affects mite longevity (van Rijn & Tanigoshi, 1999), presumably through nutritional effects. These combined strands of evidence provide considerable support for the role of extra-floral nectar as an attractant for predators of herbivorous insects, which may play a role in *E. culicivora's* attraction to *R. communis*.

However, *Lantana camara* does not have EFNs. Nevertheless, it is possible that *L. camara's* odour is attractive to *E. culicivora* for much the same reason as EFNs serve to attract predators. Other studies have shown that damaged plants that do not have EFNs produce different chemical bouquets from undamaged plants (Weissbecker *et al.*, 2000; Hammack, 2001), so it possible that the odour of the flowers of *L. camara* in olfactometer tests (which were cut at the stem, in effect

damaging the plant) was attractive to *E. culicivora* because this plant has a specific chemical signal indicating that it is damaged and that this signal is attractive to *E. culicivora*.

Chemicals that are used by plants and animals to convey information to heterospecifics are called allelochemicals. Allelochemicals are subdivided according to the costs and benefits associated with the sender and the receiver of the chemical information. There are three major types of allelochemicals (Dicke & Grotsal, 2001; Schulz, 2001), allomones, kairomones and synomones (Box 1).

Box 1. Types of allelochemicals according to the costs and benefits of the signal to the sender and the receiver.

Allelochemical category	Benefiting party	Examples
Allomones	The sender	Skunks emitting repellent odourants.
Kairomones	The receiver	Compounds given off by a prey species that attract a predator, such as the alarm pheromones given off by the ant *Iridiomyrmex pupureus* (which, as pheromones, are beneficial to ants as alarm indicators) which are used by the ant-eating spider *Habronestes bradleyi* to find its prey (Allan *et al.*, 1996).
Synomones	The sender and the receiver	The scent of flowers which attracts pollinators. The sender benefits from the potential to cross-pollinate with other visited flowers of the same species and the receiver benefits by obtaining nutrition in the form of pollen and nectar.

L. camara may be manipulating *E. culicivora* by sending a chemical signal that is beneficial to the plant but not to the spider. That the signal is an allomone, however, seems unlikely since *E. culicivora* feeds on nectar from this plant.

There is the possibility that *E. culicivora* is a pollinator of *L. camara*, in which case its odour would qualify as a synomone; but this explanation seems unsatisfactory if, as in other parts of the world, butterflies are *L. camara's* principal pollinators (Schemske, 1983; Weiss, 2000). Nevertheless, it is also possible that a damaged plant under herbivore attack that sent a signal that attracted a predator would have beneficial effects on both the plant and the predator, in much the same way as EFNs attract predators.

Finally, *L. camara's* odour may function as a kairomone. Nectar from *L. camara* may have beneficial effects on *E. culicivora*. Pollard *et al.* (1995) showed increased longevity in male *M. formosipes* spiders given access to nectar and suggested that nectar feeding may have evolved because of the selective advantage of increased longevity. Vogelei & Greissl (1989) and Taylor & Foster (1996) both showed that spiderlings given access to a simulated nectar source (i.e., a sucrose solution), survived longer than spiders given access to water alone.

Clearly, the beneficial or detrimental effects on both sender and receivers of this system remain to be clarified for the ultimate causation of this association to be discovered. *L. camara's* signal may simultaneously be a kairomone and a synomone.

An intriguing note is that studies in Uganda (McCrae *et al.*, 1968; 1969; 1976; reported in Clements, 1999) have shown that culicine and anopheline mosquitoes primarily fed from three plant species, including *L. camara.* Gary & Foster (2001, 2004) and Impoinvil *et al.* (2004) independently established that the nectar from *Ricinus communis*, and, to a lesser extent, *Lantana camara*, extends the lifespan of *An. gambiæ*. Furthermore, it has been suggested that *L. camara* may be a haven for anopheline mosquitoes (Gujral & Vasudeval, 1983; reported in Day *et al.*, 2003). Whether *E. culicivora* may be attracted to *L. camara* because of the presence of prey on the plant will be considered in Chapter 10 in light of the effect of the odour of this plant on *E. culicivora's* prey-choice behaviour.

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CHAPTER NINE
The effect of the odour of Lantana camara *plants on the prey-choice behaviour of* Evarcha culicivora

Abstract

The way in which plant odour affects a salticid's prey-choice behaviour is investigated using small juveniles of *Evarcha culicivora*, an East African salticid that feeds indirectly on vertebrate blood by choosing as prey female mosquitoes have had a blood meal. Earlier studies showed that the small juveniles of *E. culicivora* specifically choose the blood-fed females of mosquitoes in the genus *Anopheles* and that *E. culicivora* is attracted to the odour of *Lantana camara*, a plant that is common in *E. culicivora's* environment. The present study shows that the prey-choice behaviour of *E. culicivora* is altered by the odour of *Lantana camara* flowers so that, in effect, prey discrimination is eliminated by plant odour. Of three compounds found in the leaves and flowers of *L. camara* (α-(+)-pinene, β-pinene and β-caryophyllene) that were tested, only β-caryophyllene induced the same behavioural change as seen with the *Lantana* flowers. Prey-choice testing was carried out using lures made from dead mosquitoes and by using virtual lures.

Introduction

In the last chapter (Chapter 8) it was shown that *Evarcha culicivora* chooses the odour of a particular plant, *Lantana camara*, in preference to that of a sympatric plant, also found in *E. culicivora's* Lake Victoria habitat. *E. culicivora's* attraction to *L. camara* (Fig. 1) is noteworthy because it is the only spider to have a reported preference for a particular plant species. In nature, *E. culicivora* is often found on *L. camara* (RRJ, pers. comm.). It is possible that *E. culicivora* is attracted to *L. camara* because of the potential it offers for locating food sources. *L. camara's* nectar is a potential food source, both for *E. culicivora* and for other arthropods, including *E. culicivora's* preferred prey, mosquitoes (Chapter 3). Mosquitoes may feed on nectar from the flowers of this plant (McCrae *et al.*, 1968; 1969; 1976; reported in Clements, 1999; Gujral & Vasudeval, 1983; reported in Day *et al.*, 2003). Hence, the association between *L. camara* and aspects of the predatory behaviour of *E. culicivora* was chosen for investigation.

Figure 1. Male *E. culicivora* on *Lantana camara*. Photo courtesy of Robert Jackson.

Previous studies showed that *E. culicivora* distinguishes between different mosquito types based on visual cues alone (Chapter 3, 4, 5), and chooses certain types of mosquitoes. I decided to investigate whether this prey-choice behaviour was affected by odour cues associated with *L. camara*, in particular, by the presence of odour from *L. camara* flowers or by volatiles (α-(+)-pinene, β-pinene and β-caryophyllene) produced by *L. camara*.

Materials and Methods

General

Studies were conducted at the Spider Laboratory at the University of Canterbury in New Zealand. All living spiders came from laboratory culture, and standard spider-laboratory procedures were adopted (Jackson & Hallas, 1986). All testing was carried out between 0700 h and 1200 h.

All test spiders had been starved for 5-7 days before testing. No spider was used in more than one test per day and no spider was used twice in the same experiment.

Testing with lures

Each individual predator was simultaneously given access to two lures, each made from a blood-fed female mosquito (body length, 4.5 mm). Methods for making lures of mosquitoes were the same as those used in Chapter 3. One lure was made from *Anopheles gambiæ* and the other was made from *Culex quinquefasciatus*. These lures were presented to spiders in the presence of the odour of *Lantana camara* flowers and in the absence of flower odour. All spiders used in tests were juveniles of *E. culicivora* (body length, 1.5-2.0 mm).

Each lure was placed at one of the two ends of a Y-shaped ramp that was angled up 20° (see Fig. 2). This method for the simultaneous-presentation of lures was designed specifically to test juvenile spiders. With minor improvements, the methods used here were similar to those used in Chapter 4. The interest of the spiders was maintained by movement of the lures and the ramp was small enough so that spiders would not be further than about 20 body lengths away from the lure at the beginning of the test (20 body lengths being the distance other studies have established as the distance at which discrimination between prey and non-prey is readily achieved; Harland *et al.*, 1999). The test apparatus was made from varnished wood that was wiped with 80% ethanol after each test to eliminate the possibility of chemical traces from previous tests that might affect the outcome of future tests.

Each of the two lures was attached with double-sided tape to the stem of a seconds-hand (i.e., where the seconds hand originated) from a battery-operated clock. The actual 'hand' (i.e., the stick) had been cut off the base from which it stemmed. By using the circular stem of a seconds-hand as the pedestal on which lures were placed, lures moved one notch per second (360° per min). Each clock mechanism was firmly glued under each arm of the ramp (i.e., the lures were at the upper end of the ramp) and the stem for the seconds hand lay directly above the ramp so that lures were visible above the ramp. Each clock was individually wired to an external controller so that it could be turned on and off. Lures were rotated for *c.* 30 s about once every 3 min before and during the test. Movement at the beginning of the test was done in order to attract the attention of the salticid and movement during the test helped maintain the salticid's interest in the lures (preliminary testing indicated that juveniles of *E. culicivora* were disinclined to respond to motionless lures). Lures were not kept rotating permanently because of the possibility of deterring juvenile spiders from attacking their choice of prey. After each movement period, the lure was positioned so that the mosquito was oriented diagonally (45°) relative to the stem of the ramp (this was achieved because a full 360° rotation takes 60 s; consequently every 30 s the lure rotated 180°, and, if placed at a 45° angle to begin with, the lure rotated between 45° and 225°, and vv, in each 30 s movement period throughout the test). In this way, the salticid could see the lure's head as well as its abdomen. Unlike other ramp designs used in salticid studies (e.g., Chapter 4), this ramp did not have a wooden backdrop against which *E. culicivora* saw the lures.

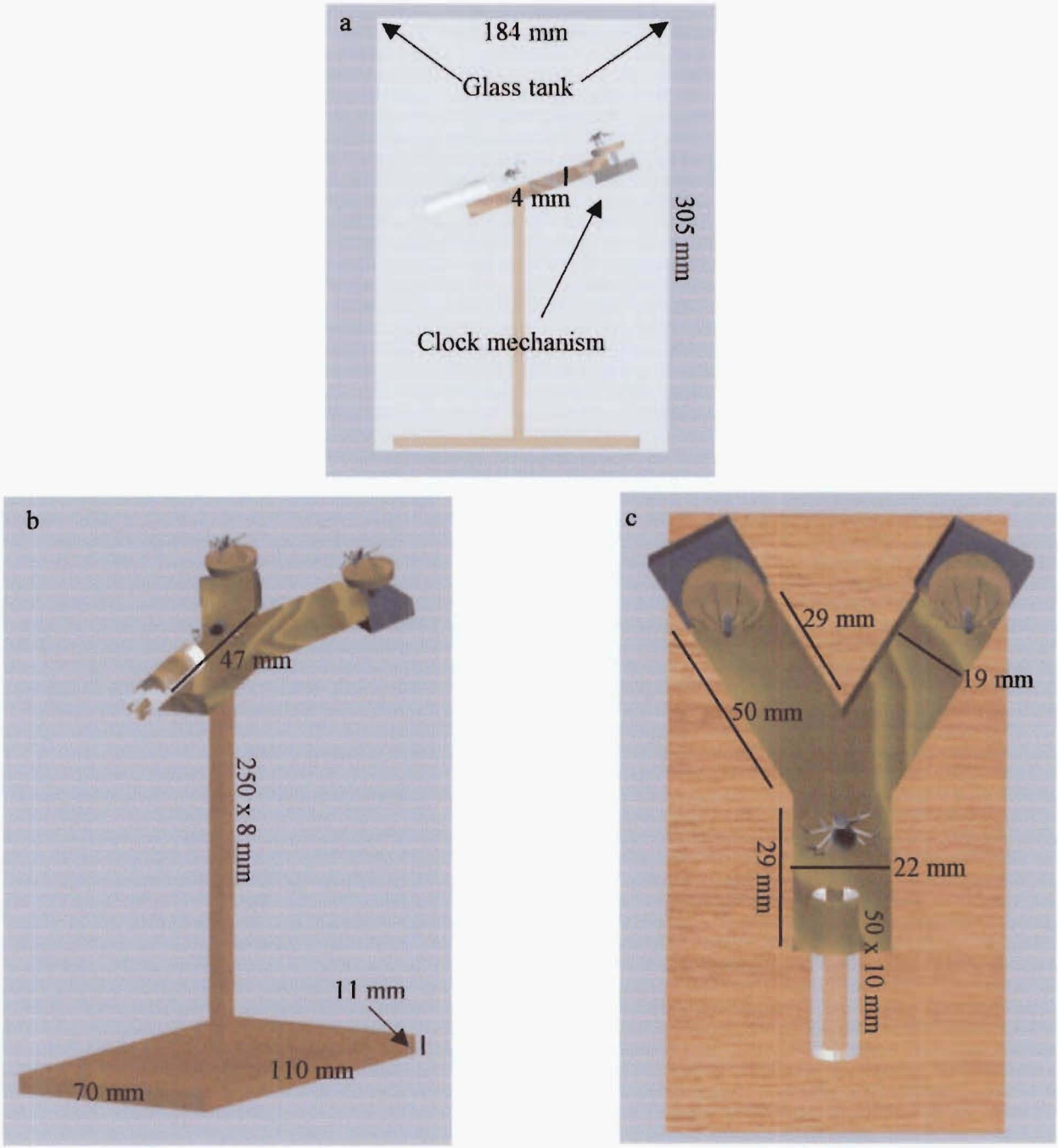

Figure 2. Y-shaped ramp (at 20° angle) used in experiments with lures (not to scale). Clock-control wires, mesh and odour source not shown. **a)** Side-on view of experimental set-up showing glass tank in which tests were held. **b)** Perspective view of ramp. Dimensions of platform and dowel shown as well as distance from stem to Y junction. Glass tank not shown. **c)** Ramp seen from above. Dimensions of stem, arms and preserving vial from which spiders began tests are shown. Glass tank not shown.

A plastic vial (50 mm in length X 10 mm diameter) packed with cotton to about 5 mm from the open end was stuck on the stem of the ramp (with double-sided tape). Tests began when a spider, which had been placed on the cotton within the vial, walked out of the vial and on to the ramp. The cotton was changed after each test to eliminate chemical cues from draglines of previously tested spiders that might affect the outcome of subsequent tests.

All tests took place within a glass tank (178 mm wide, 305 mm long and 184 mm deep) standing on end (i.e., the top, or open, end of the glass tank was vertical and faced the experimenter). The open side of the glass tank was covered with a second piece of glass which was taped onto the tank to hold it upright. This second piece of glass, the 'door', was placed so that it left only a small gap at the bottom through which the control wires leading from the clocks were threaded. All sides of the glass tank had been covered with white paper (taped on the outside). This paper prevented the spider being distracted by movement from the experimenter and provided a background against which the spider could see the lures.

Fine mesh (*c.* 0.5 mm holes) that had been folded over three times was taped to the walls of the glass tank, about half way up (i.e., *c.* 150 mm from the bottom). The mesh allowed the odour of *Lantana camara* inflorescences to permeate the experimental area above the mesh while preventing *E. culicivora* from seeing the plant underneath. There were two types of test, those with plant odour and those without plant odour. Tests in which there was plant odour were carried out by placing a small plastic pottle with water, inside which were two *L. camara* flower heads (with stems, brachts and two leaves), at the bottom of the glass tank, underneath the mesh, 5 min before each test. The flower-containing pottle was in this location throughout the test. Experiments without plant odour were identical except that, in this case, the pottle with water did not have *L. camara* flowers in it.

Test duration was 45 min. Unsuccessful tests were those in which: the spider, having left the vial, failed to move up to within 10 mm of the lure within the 45 min test-period; the spider had not left the vial after 5 min; the spider jumped off the ramp. Successful tests were those in which spiders walked up the ramp to within 10 mm of one or the other lure and stayed there for longer than 30 s, or when the spider walked up to and jumped on one of the lures. Results were analysed using chi-square tests of goodness of fit and chi-square tests of independence (Sokal & Rohlf, 1995).

Testing with compounds found in the headspace of *Lantana camara*

The principal components in the headspace of *Lantana camara*, regardless of the origin of the plant, include α-(+)-pinene, β-pinene and β-caryophyllene (da Silva *et al.*, 1999; Ngassoum *et al.*, 1999;

Kahn *et al.*, 2002; Sefidkon, 2002). Although always present in high amounts, the relative amounts of these three compounds differed in different studies. Other components of the leaves and flowers (and headspace) of *Lantana* included: limonene; α-phellandrene; germacrene-D; sabinene; ar-curcumene; caryophyllene epoxide; davanone; linalool; γ-elemene; β-elemene; α-copaene; α-cadinene; bicyclogermacrene and α-hamulene, but the relative quantities and presence of these other compounds differed between studies, which were carried out on *Lantana* plants from Iran, India, Brazil, Cameroon and Madagascar.

Tests on *E. culicivora* were carried out using α-(+)-pinene, β-pinene and β-caryophyllene. The samples of these compounds were not pure to over 90% as is the norm in the literature. The aim of these experiments was to see whether there was a change in prey-choice behaviour of *E. culicivora* in the presence of vapour from these three compounds.

Virtual mosquitoes were derived from blood-fed females of *Anopheles* and were animated to be grooming intermittently (for drawing and animation methods, see Appendix I). Because results from another study (Chapter 7) showed that *E. culicivora* chooses virtual mosquitoes more often when the mosquito's abdomen is red than when it is black, the abdomens were red and the heads and thoraces were in 'greyscale' (black and white). Test spiders were juveniles (body length, 1.5 mm). During tests, two virtual mosquitoes were placed side by side. They differed only in posture. One mosquito was placed in an anopheline posture, with its abdomen at angled up at 45°, and the other mosquito was placed with its abdomen in a horizontal posture (both mosquitoes were 3.2 mm long). Whether the mosquito in the tilted position was on the left-hand side of the screen or the right-hand side of the screen was randomised (animations were made for both sides of the screen). In previous tests without odour (Chapter 5), 1.5 mm juveniles exhibited a 9:1 preference for the mosquitoes in the tilted posture. Deviation from this ratio was used as a measure to determine if prey-choice behaviour was altered by α-(+)-pinene, β-pinene or β-caryophyllene.

In tests with adults, only β-caryophyllene was used. In these tests, the virtual prey that were presented to the salticids were side-on views of two blood-fed mosquitoes with red abdomens that differed in that one had male, not female, antennae (for drawing and animation methods, see Appendix I). As in other tests with virtual prey, the position of the virtual mosquitoes was randomised. Both virtual mosquitoes were 3.2 mm long. In previous tests without odour (Chapter 4) adults of *E. culicivora* exhibited a 3:1 preference for the mosquitoes with female antennae. Deviation from this ratio was used as a measure to determine if prey-choice behaviour was altered by β-caryophyllene.

Methods for projection were similar to those used in Chapter 4, with modifications being made in order to present spiders simultaneously with odour cues and visual cues. The apparatus was a glass cylinder (525 mm long x 300 mm in diameter) with removable steel end-plates (200 mm diameter) that were screwed in place and attached with butterfly nuts (6 per end-plate) (Fig. 2). A lens that reduced the projected image was snugly fitted into a hole (diameter 37 mm) in one end plate (this was not a reducing lens but a magnifying lens, which had the effect of reducing the projected image). The size of the projected virtual mosquitoes was calibrated by altering the size of the window in which the animation was playing on the computer (until the mosquitoes, in reduced form, measured 3.2 mm on the screen on to which they were projected). The 'reducing' lens was aligned with the lens of a data projector. The end-plate containing the reducing lens was removed for cleaning and for airing-out the cylinder when a complete series of tests with one particular chemical had ended. The other end-plate was removed for cleaning the cylinder and for inserting the test spider before each test.

Within the cylinder there was a stainless steel platform (150 mm wide) that spanned the entire length of the cylinder (Fig. 3) on which a ramp was fitted. The stainless steel ramp was 29 mm wide and 95 mm long. The image of the virtual prey was projected on a fine-ground matte unmarked type D Nikon F3 focusing screen (39 mm wide x 30 mm high) mounted in a specially designed bracket. This bracket was welded to a thin piece of stainless steel that was itself attached to the ramp (the 'flap'). By moving the stainless steel 'flap', the focusing screen could be positioned so that it was closer (2 mm) or further (5 mm) from the ramp. The entire unit of the ramp-and-focusing-screen was placed so that the focusing screen was located at a distance of *c.* 105 mm from the reducing lens in the end-plate of the cylinder. The ramp-unit had two (*c.* 5 mm diameter) stainless steel 'legs' which slotted into holes in the steel platform. In this way the ramp-unit could not move and the distance between the focusing screen and the reducing lens was always the same (±3 mm variation in the position of the focusing screen). By placing the reducing lens directly in front of the projector lens, the projected image was constant for all tests. For all tests with juveniles, the focusing screen was placed so that it was 2 mm in front of the ramp.

At a distance of 22 mm from the end of the ramp, there was a stainless steel 'starting box' (11 mm wide X 19 mm high X 22 mm deep; i.e., deep end 44 mm from top end of ramp) which was welded to the ramp. The box had a transparent Perspex cover which was wired up to an external controller so that it could be opened (with a spring-opening system) remotely by pressing a button.

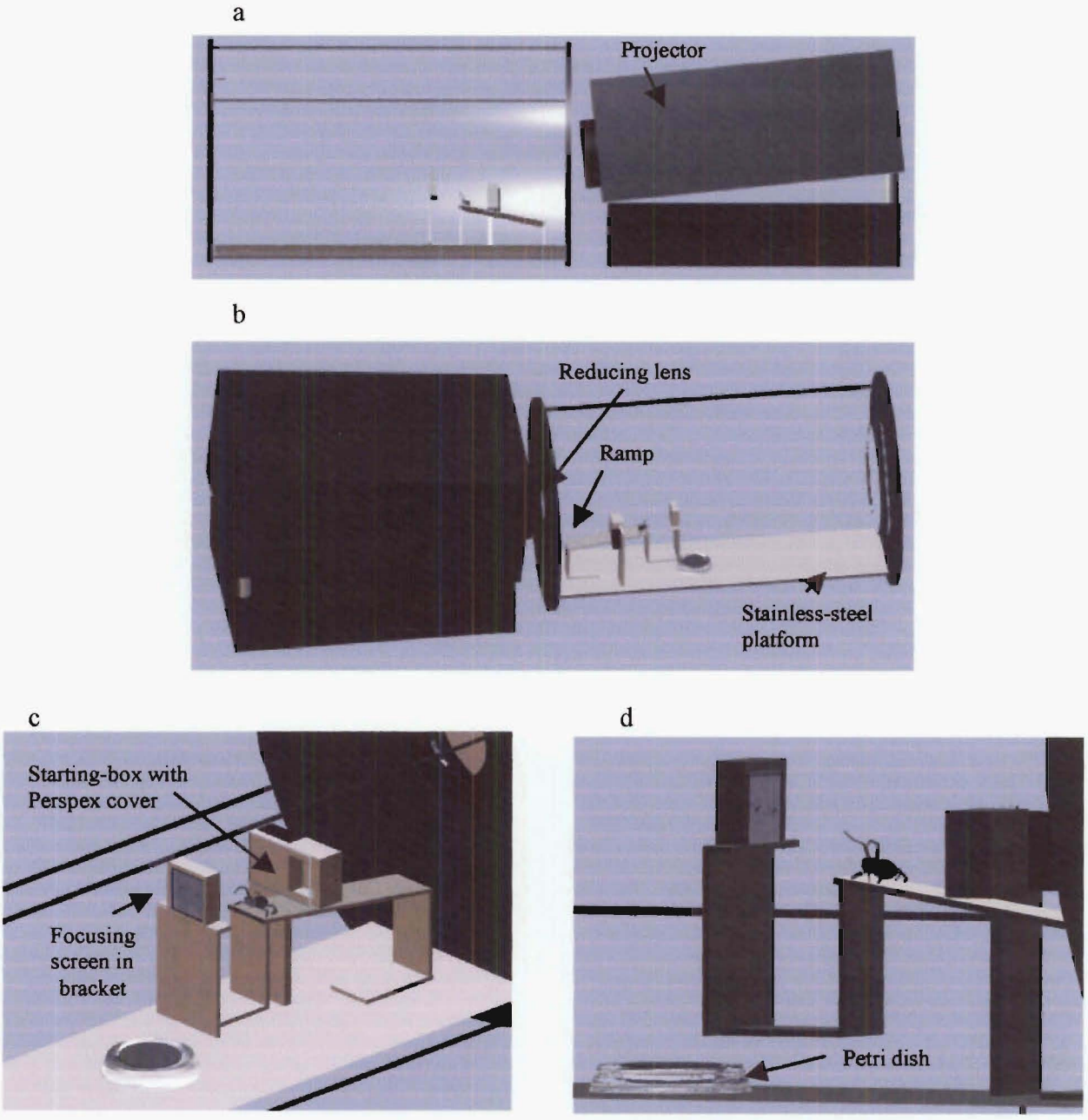

Figure 3. Methods used for presenting spiders with animated virtual prey in the presence of odour. **a)** Side-on view of the experimental apparatus and data projector used in tests in which spiders were presented with virtual prey and with odour. **b)** Perspective view of experimental apparatus and projector showing how the projected image goes through a reducing lens fitted in a hole within the end-plate. **c)** Close-up view of ramp showing the mounting bracket into which the focusing screen is fitted, the starting box with the Perspex cover and the position of the petri dish with the filter paper containing chemical compounds. **d)** View from behind the spider illustrating the position of the petri dish relative to the focusing screen. Note: spider not to scale.

Using a soft-haired paint-brush, adult spiders were placed in the starting box. The ramp was then put in place, the chamber was sealed, and the test began when the door of the starting box was opened (after *c.* 60 s). Juveniles of *E. culicivora*, being much smaller (*c.* 1.5 mm) than adults, were placed closer to the screen, so that they were at a comparable distance from the screen to adults (8-10 body lengths away from the screen). Consequently juveniles were not placed in the starting box before testing, as done with adults. Instead, as in previous studies (Chapter 5), juveniles were placed on the ramp, using a soft-haired paint-brush, at about 10 mm from the end of the ramp. *E. culicivora* is a 'calm' salticid that will sit peacefully on a brush. Tests with juveniles began as soon as the spider walked off the brush and on to the ramp. Immediately after the spider had walked off the brush, the steel end-plate was replaced and screwed in place. The animation sequence used in tests was already being played before the spider was placed on the ramp.

The data projector was positioned angling down *c.* 10° (see Chapter 4), and the testing ramp was angled up *c.* 25° toward the focusing screen. With these angles, shadows were avoided and the stalking spiders did not obscure the projected image.

Tests lasted 15 min, but, if stalking had begun, tests were extended until the end of the stalking bout (this was never more than 18 min). Stalking is defined as the behaviour of the spider once it had oriented toward the prey and had begun approaching in a direct line while it waving its palps (toward and away from its face) in matching phase (see Chapter 6). The spider's approach toward the prey (which had a 'purposeful' appearance to it) tended to slow down as the spider got nearer to it.

After each test, the ramp was taken out of the cylinder and wiped with 80% ethanol to eliminate traces of chemicals that might influence the outcome of subsequent tests. Criteria for successful tests were identical to those described for other virtual-prey choice experiments with juveniles (Chapter 4, 5). Unsuccessful tests were those in which the spider walked away from the projected image, tests that lasted longer than 15 min without the spider walking toward the virtual mosquitoes and tests in which the spider jumped off the ramp or failed to move.

For each test, 4 µl (to nearest 0.2 µl) of the compound in question was deposited onto a disc of filter paper (diameter 42 mm) inside a 50 mm glass petri dish using a 10-µl syringe. This petri dish was placed on the stainless steel platform directly behind the focusing screen (out of the spider's line of sight) (Fig. 3). The experimental chamber (the cylinder) was preincubated with odour by placing the odour source (petri dish) in the cylinder for 15 min before the first test. The petri dish was left in the cylinder for the duration of the test. After the first test, and for every

subsequent test, the filter paper was replaced with a new piece on which a new 4 µl sample of the compound was deposited. In this way the cylinder had odour throughout all tests in a day. After each chemical had been tested (i.e., when there was to be a change of chemical compound), the entire apparatus was dismantled (both end-plates were removed), cleaned with 80% ethanol and allowed to dry for 24 h before tests with the next chemical were initiated.

Results were analysed using chi-square tests of goodness of fit, chi-square tests of independence and Fisher exact tests (Sokal & Rohlf, 1995).

Results

Testing with lures

Odour from *L. camara* flowers affected the prey-choice behaviour of the small juveniles of *E. culicivora*. In the absence of odour from *L. camara* flowers, *E. culicivora* chose lures made from *Anopheles gambiæ* mosquitoes (72%) somewhat more often than they chose lures made from *Culex quinquefasciatus* mosquitoes (Fig. 4), although this was not quite significant (χ^2=3.56, P=0.059, N=18). In the presence *L. camara* flower odour, no choice by *E. culicivora* between the lure made from *An. gambiæ* (38%) and the lure made from *C. quinquefasciatus* (Fig. 4) (χ^2 =1, NS, N=16) was evident.

There was a significant difference between the prey-choices made by *E. culicivora* toward the lures in the presence or absence of flowers (χ^2=4.14, P<0.05, N=34). In general, *E. culicivora* chose *Anopheles* more often than *Culex* in the absence of odour of *L. camara* flowers, but not the presence of flowers.

There was no evidence that the presence of odour affected the number of successful tests (Fig. 5). The number of failed tests in the presence of odour did not differ significantly from the number of failed tests in the absence of odour (P=0.29, NS, N=41).

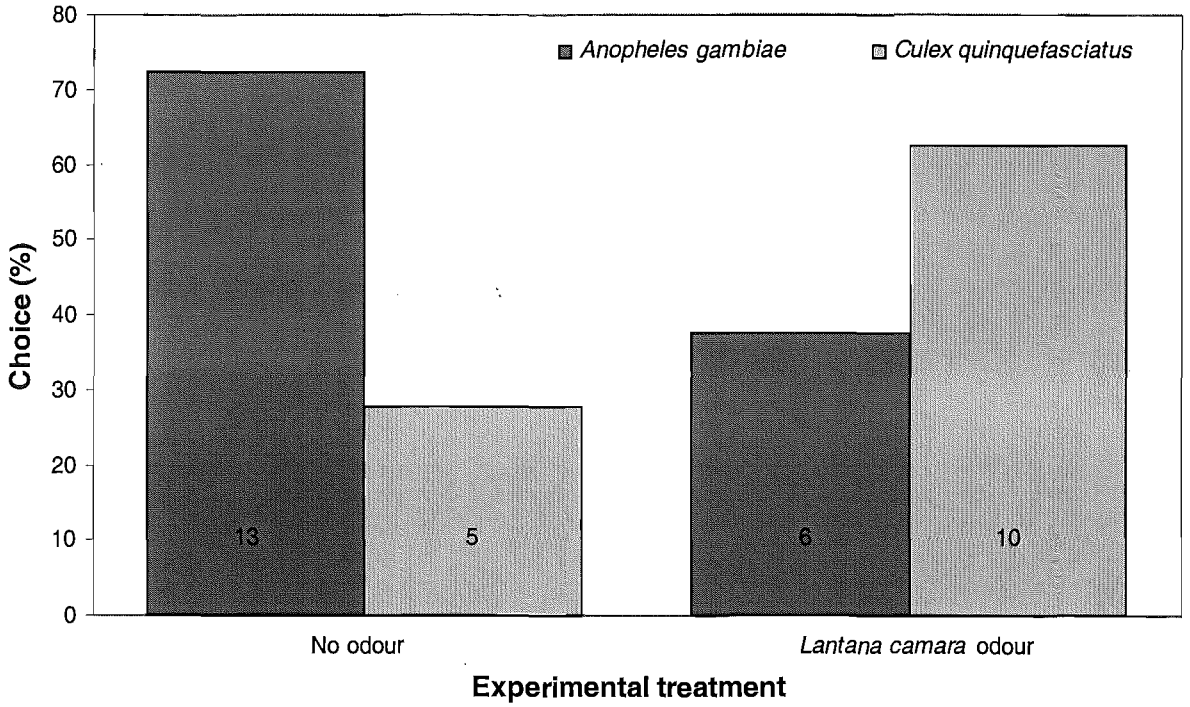

Figure 4. Percentage of juveniles of *Evarcha culicivora* (body length, 1.5-2.0 mm) that chose different mosquito prey (*Anopheles gambiæ* and *Culex quinquefasciatus*) in the absence of odour from prey but with and without the odour of *Lantana camara* flowers. Test spider had simultaneous access to lures made from two blood-fed female mosquitoes of the same size (4.5 mm). Numbers of spiders that went towards each lure shown within each bar.

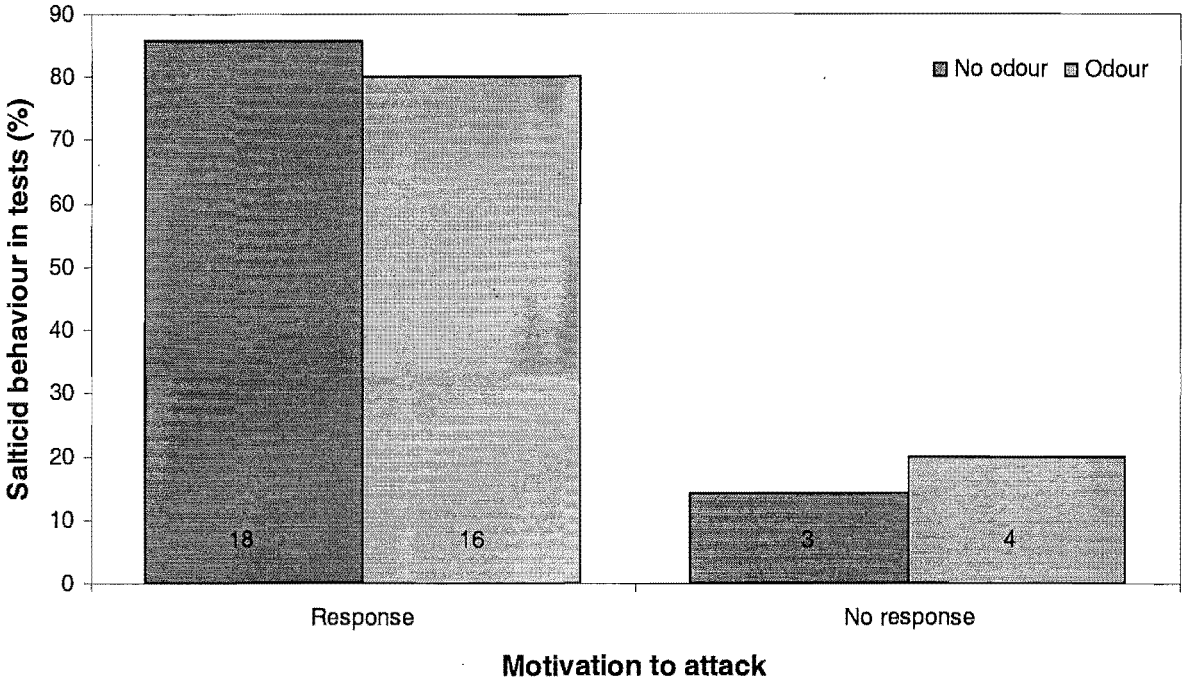

Figure 5. Overall percentage of juveniles of *Evarcha culicivora* (body length, 1.5-2.0 mm) that responded to lures made from dead mosquitoes (*Anopheles gambiæ* and *Culex quinquefasciatus*) in the presence and absence of odour of *Lantana camara* flowers. N shown within each bar.

Testing with compounds found in the headspace of *Lantana camara*

When α-(+)-pinene (χ^2=5.33, P<0.05, N=12) or β-pinene (χ^2=6.41, P<0.05, N=10) vapours were present in the apparatus, findings were comparable to the findings in Chapter 5 (Fig. 6). With these two compounds, there was no discernible effect on the prey-choice behaviour of juvenile *E. culicivora.* (i.e., juveniles of *E. culicivora* chose virtual mosquitoes that were in an *Anopheles* posture (with the abdomen tilted at an angle) more often than they chose mosquitoes in a horizontal posture). However, in the presence of β-caryophyllene vapour, *E. culicivora* showed no tendency to choose one virtual prey more often then the other (χ^2=0, NS, N=12) (Fig. 6). The choices made in the presence and absence of β-caryophyllene were significantly different (P<0.02, N=32), but the choices made in the presence of α-(+)-pinene (P=0.86, NS, N=32) and β-pinene (P=0.75, NS, N=30) did not differ significantly from the choices made without odour (Fig. 6).

The number of failed tests in the presence of α-(+)-pinene odour (P=0.13, NS, N=40) and β-caryophyllene odour (P=0.47, NS, N=45) did not differ significantly from the number of failed tests in the absence of odour (Fig. 7). However, the number of failed tests in the presence of β-pinene odour was significantly lower than in its absence (P<0.05, N=39) (Fig. 7).

In tests with adults in the presence of β-caryophyllene vapour, results were different from those in Chapter 4. In previous experiments (Chapter 4), adults chose the virtual mosquitoes with female antennae more often than mosquitoes with the male antennae. In these tests, using the same virtual prey, but in the presence of β-caryophyllene vapour, the number of adults of *E. culicivora* that chose the mosquito with female antennae (40%, n=8) was not significantly different from the number of spiders that chose the mosquito with male antennae (60%, n=12), corroborating the effect of β-caryophyllene on *E. culicivora's* prey-choice behaviour. When choices in the presence and absence of β-caryophyllene were compared, it was apparent that β-caryophyllene had a significant effect on *E. culicivora's* prey-choice behaviour (χ^2=5.01, P<0.05, N=40).

The number of failed tests in the presence of odour did not differ significantly from the number of failed tests in the absence of odour (P=0.60, NS, N=49). In the absence of odour, there were 23 successful and 3 unsuccessful tests with adults. In the presence of β-caryophyllene, there were 20 successful and 3 unsuccessful tests with adults.

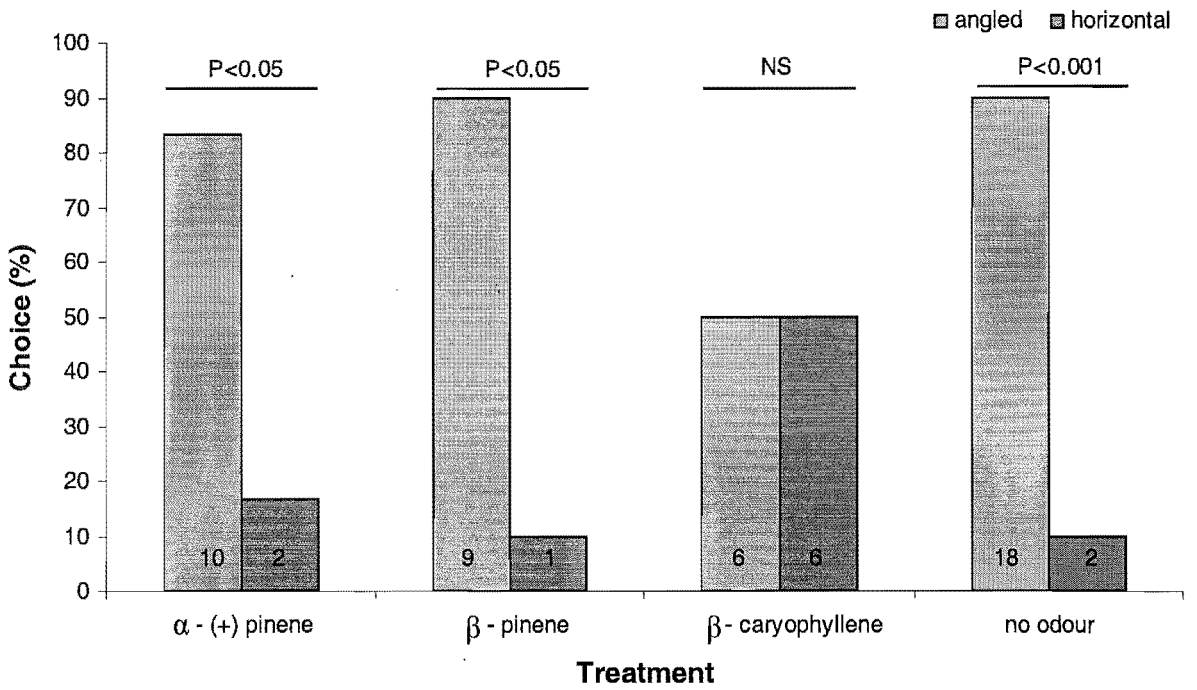

Figure 6. Percentage of juveniles of *Evarcha culicivora* (body length, 1.5 mm) that chose virtual mosquitoes in different postures in the presence of volatile compounds found in the headspace of *Lantana camara* plants. Four treatments: α-(+)-pinene, β-pinene, β-caryophyllene and no odour. Test spider had simultaneous access to two virtual lures based on blood-fed females of *Anopheles gambiæ* mosquitoes. All choice tests with identical mosquitoes that differed in resting posture. One prey: abdomen horizontal (resting posture of *Aedes* and *Culex* mosquitoes). Other prey: abdomen tilted up at a 45° angle (resting posture of *Anopheles* mosquitoes). Virtual mosquitoes same size (3.2 mm). Numbers of spiders that chose each prey shown in bars. Chi-square tests of goodness of fit (null hypothesis: choose each prey type equally often).

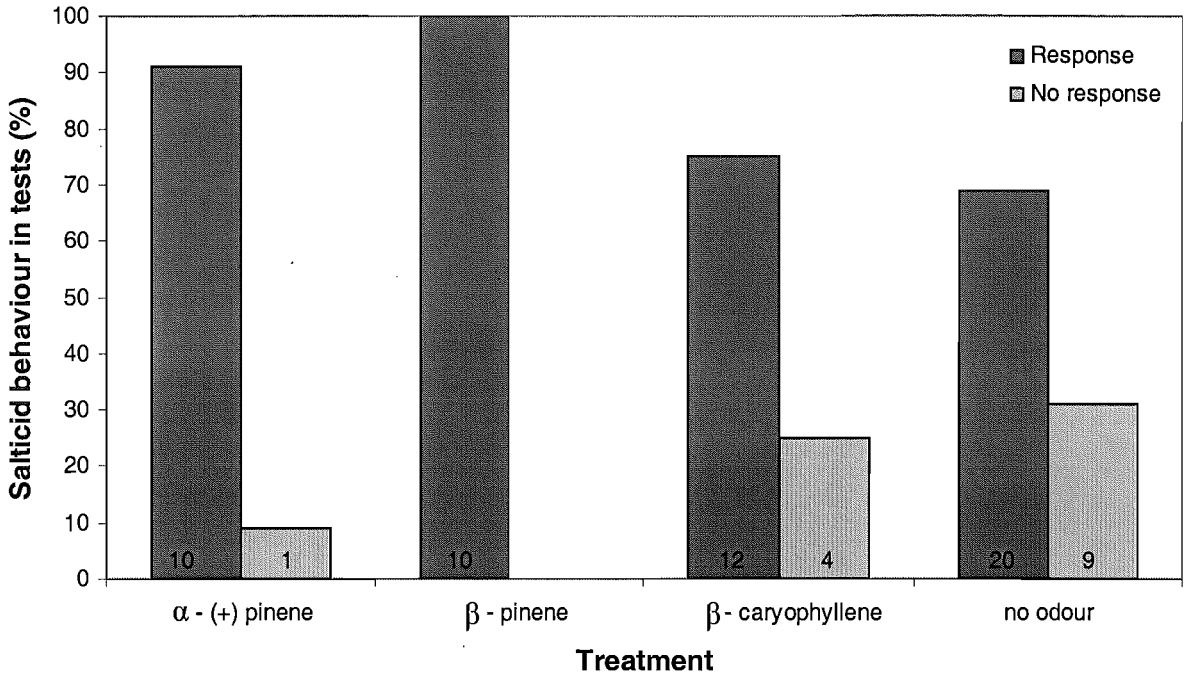

Figure 7. Overall percentage of juveniles of *Evarcha culicivora* (body length, 1.5 mm) that responded to virtual lures (mosquito resting in horizontal position and mosquito resting in tilted position typical of *Anopheles*) in the presence and absence of α-(+)-pinene, β-pinene and β-caryophyllene. N shown within each bar. Chi-square tests of independence (null hypothesis: responded to lures equally often regardless of treatment). Odour had no effect on motivation to attack lures, except with β-pinene where the response of spiders toward lures was significantly higher ($P<0.05$) in the presence of the compound than with no odour.

Discussion

The results from this study indicate that *Evarcha culicivora's* prey-choice behaviour changes in the presence of the odour from *Lantana camara*, a plant to which this spider is attracted (Chapter 8) and on which it is often found (RRJ, pers. comm.). Furthermore, the finding that β-caryophyllene is a proximate cause in the behavioural change of *E. culicivora* is of considerable interest. The connection of a significant behavioural change to a single environmental chemical stimulus for spiders is a novel and important finding.

E. culicivora is a selective predator; it chooses blood-fed female mosquitoes as its preferred prey. Small juveniles of *E. culicivora* have more precise prey-choice behaviour; they prefer mosquitoes belonging to the genus *Anopheles* (Chapter 5). Yet, in the presence of the odour of the plant to which they are attracted, *L. camara*, and in the presence of a volatile produced by *L. camara*, β-caryophyllene, *E. culicivora* becomes indiscriminate in its prey choice. β-Caryophyllene is a sesquiterpene that is found in a wide variety of flowering plants (da Silva *et al.*, 1999; Weissbecker *et al.*, 2000; Khan *et al.*, 2002; Araujo *et al.*, 2003) and is known to be attractive to several insects (Weissbecker *et al.*, 1999; Zhu *et al.*, 1999; Al Abassi *et al.*, 2000; Kalinová *et al.*, 2000; van Loon *et al.*, 2000; Hammack, 2001; Bichão *et al.*, 2003; Syed & Guerin, 2004).

At first glance, the behavioural change caused by β-caryophyllene in *E. culicivora* may appear detrimental to the spider and raises the question why *E. culicivora* should be attracted to a plant that has a 'psychological' effect on *E. culicivora* that makes it less discriminating about its prey ('spider gone mad') when usually *E. culicivora* is particularly selective about its prey (Chapter 3, 5). A closer look at the association between *E. culicivora*, its mosquito prey and *L. camara* may suggest that, despite the behavioural change, the ability to distinguish *L. camara* on the basis of odour may have a positive impact on *E. culicivora*.

L. camara may be a potential nectar source, both for *E. culicivora* and for *E. culicivora's* preferred prey, mosquitoes (Chapter 3). Salticids are known to feed on nectar (Ruhren & Handel, 1999; Jackson *et al*, 2001), but *E. culicivora* is unique because it is the only spider for which an odour preference for a certain plant has been established (Chapter 8). There is also some evidence that *L. camara* may be attractive to mosquitoes. Studies to date have emphasized the semiochemical detection of hosts by insect vectors, particularly the principal malaria vector in Africa, *Anopheles gambiæ* (e.g., de Jong & Knols, 1995; Constantini *et al.*, 1998; Takken & Knols, 1999; van den Broek & den Otter, 2000; Fox *et al.*, 2001; Meijerink et al., 2001; Murphy *et al.*, 2001; Merrill *et al.*,

2002), but little work has been done on the important question of what these vectors do when they are *not* feeding on blood.

Mosquitoes use flowers as feeding sites (Clements, 1999). Male mosquitoes do not feed on blood. Instead, they survive by feeding on nectar from plants. Female mosquitoes also feed on nectar because it provides the mosquito with an immediate energy source (Clements, 1999) that can be used in the initial host-seeking flight (Takken & Knols, 1999; Foster & Takken, 2004) in search of a blood meal. *Lantana camara* may be a favoured plant from which to feed. Studies in Uganda (McCrae *et al.*, 1968; 1969; 1976; reported in Clements, 1999) found culicine and anopheline mosquitoes primarily fed from three plant species, including *L. camara.* Gary & Foster (2004) and Impoinvil *et al.* (2004) independently established that the nectar from *Ricinus communis*, and, to a lesser extent, *L. camara*, extends the lifespan of *An. gambiæ*. These results suggest that the interaction between *L. camara* (and *R. communis*) and mosquitoes in nature may be significant. Furthermore, a study in India reported that *L. camara* harbours malarial mosquitoes (Gujral & Vasudeval, 1983; reported in Day *et al.*, 2003).

L. camara is a haven for other disease vectors in Sub-Saharan Africa, notably tsetse flies, the vector of sleeping sickness (Leak, 1998). There is evidence that tsetse flies not only use semiochemicals to respond to olfactory stimuli from their vertebrate hosts but also use semiochemicals to locate plants that provide suitable cover (Syed & Guerin, 2004). In particular, tsetse flies are attracted to *L. camara* and detect it using the compounds β-caryophyllene and 1-octen-3-ol, for which they have specific receptors on their antennae (Syed & Guerin, 2004). This is particularly noteworthy because the results presented in this chapter indicate that β-caryophyllene is a proximate cause in *E. culicivora's* behavioural change in the vicinity of *L. camara*. Whether β-caryophyllene is the active ingredient that is attractive to *E. culicivora* when searching for *L. camara* is currently being studied in Mbita (Kenya) (RRJ, pers. comm.). Direct evidence of *An. gambiæ's* attraction to *L. camara* is lacking, but studies are underway in Kenya (RRJ, pers. comm.) exploring both this question and whether *An. gambiæ* is attracted to β-caryophyllene.

Theory predicts that animals should optimise their time searching for the nutrients they require. For an animal that has a preference for mosquitoes, searching the environment at random may not be the ideal way of finding them, if mosquitoes congregate in certain areas. As suggested, *L. camara* may provide mosquitoes with shelter and nectar and consequently may be attractive to mosquitoes, in which case, *L. camara* should be attractive to the mosquito's predator, *E. culicivora*. *L. camara* is a highly visual plant with colourful flowers (Fig. 1) that flower year-round in suitable

climates (like Lake Victoria). These flowers may serve as indicators of a productive food patch and *Lantana's* odour may provide *E. culicivora* with a long-distance signal as to the plant's whereabouts, as is assumed to occur with tsetse flies attracted to *L. camara* (Syed & Guerin, 2004). It is noteworthy that *E. culicivora* is most commonly found in the vicinity of houses and on *L. camara*. Mosquitoes that feed on human blood, such as *An. gambiæ*, congregate around humans, i.e., they enter houses in search for food. Although anecdotal, these strands of evidence suggest that *E. culicivora* 'knows' where to find productive food patches containing its preferred prey: houses and *L. camara*. The ability to detect *L. camara* is then beneficial to *E. culicivora*. In the event of not finding prey *E. culicivora* can feed on nectar.

Although *E. culicivora's* prey-choice behaviour is impaired ('spider gone mad') in the vicinity of *L. camara*, this does not negate benefits accruing to the spiders by having an increased availability of food because, as these results suggest, the spider's ability to kill prey is not impaired. In fact, at least among juveniles, there is evidence that the spider's ability to kill prey is improved on *L. camara* (RRJ, pers. comm.).

Nectar provides the small juveniles of *E. culicivora* with an energy boost that allows them to tackle large prey, such as mosquitoes (RRJ, unpublished data). Unlike larger juveniles and adults, small juveniles of *E. culicivora* do not have the mass to hold a mosquito down and typically the mosquito starts flying about with the spider hanging onto the prey until eventually the mosquito becomes subdued and falls to the substrate (Chapter 6). Nectar from *L. camara* may provide juveniles of *E. culicivora* with the extra energy needed for the effort of capturing prey several times larger and stronger than themselves. Consequently, for the juveniles, there may be the added benefit of finding prey at a location where they can obtain enough energy to capture it. That the innate preference of small juveniles of *E. culicivora* for *Anopheles* mosquitoes disappears on *L. camara* may not be particularly detrimental. Although juveniles of *E. culicivora* identify *Anopheles* by its characteristic posture (Chapter 5), this does not imply that *Anopheles* is chosen *because* of its posture. Instead, *Anopheles* may be chosen because it tends to be smaller, more manageable, prey than sympatric mosquitoes from different genera. The prey-specific predatory tactic used by small juveniles of *E. culicivora* may be an adaptation that exploits the characteristic resting posture of *Anopheles*, allowing the spiderling to 'get a good grip' on the mosquito when it bites it and the mosquito starts flying about. Mosquitoes from other genera tend to be larger than *Anopheles* and also rest with their bodies horizontal to the substrate. This position may make it harder for small juveniles of *E. culicivora* to hold onto their prey because the spider cannot get directly under the

mosquito, like they usually do with *Anopheles*, but need to leap on it. It is possible that the extra energy provided by the nectar may be sufficient to allow juveniles to tackle mosquitoes from other genera as well, in which case, the drug-induced (β-caryophyllene-induced) inability to discriminate prey (or the loss of 'caring' about which prey to attack) still does not negatively affect the spider gone mad.

Furthermore, there is also the possibility that a mixed diet (Evans *et al.*, 1999) (of male mosquitoes and blood-fed females, for example) is actually more beneficial to *E. culicivora* than a pure diet of blood-fed female mosquitoes, in which case, *Lantana* plants have yet a further beneficial impact on *E. culicivora*. In fact, evidence to date supports the notion that a mixed diet is better for *E. culicivora* than a pure diet of blood-fed female mosquitoes (RRJ, unpublished data).

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CHAPTER TEN

A psychological effect of β-caryophyllene, a volatile from Lantana camara, *on* Evarcha culicivora

Abstract

The effect of the odour from *Lantana camara* on the prey-choice behaviour of the mosquito-eating salticid from East Africa, *Evarcha culicivora*, was investigated. Prey-choice behaviour was investigated using 3D animated virtual lures of mosquitoes and of similar-looking, non-biting midges that vastly outnumber mosquitoes in *E. culicivora's* habitat. Previous studies have shown that odour of *L. camara* flowers alters the mosquito prey-choice behaviour of *E. culicivora.* This study assessed whether mosquitoes and midges could be distinguished based on optical cues in three contexts: in the absence of odour, in the presence of odour from flowers of *L. camara* and in the presence of a compound present in the leaves and flowers of *L. camara*, β-caryophyllene. The results indicate that adults of *E. culicivora* choose mosquitoes more often than midges in the absence of odour but do not choose mosquitoes more often than midges in the presence of odour from *L. camara* or from β-caryophyllene.

Introduction

Evarcha culicivora is an East African jumping spider that has a specific prey-preference for blood-fed female mosquitoes (Chapter 3). Juveniles of *E. culicivora* are even more particular about their preferred prey, choosing mosquitoes in the genus *Anopheles* in preference to mosquitoes belonging to other genera (Chapter 5). However, in the presence of *Lantana camara* plants, to which *E. culicivora* is attracted (Chapter 8), the spider's prey-choice behaviour is altered. Laboratory studies have indicated that this normally choosy spider becomes indiscriminate in its choice of mosquito prey (Chapter 9). One compound produced by *L. camara*, the sesquiterpene β-caryophyllene (Fig. 1) (da Silva *et al.*, 1999; Ngassoum *et al.*, 1999; Kahn *et al.*, 2002; Sefidkon, 2002), caused the behavioural change in *E. culicivora* (Chapter 9).

Having already shown that β-caryophyllene affects the mosquito-choice behaviour of *E. culicivora* (Chapter 9), I wanted to investigate *E. culicivora's* prey-choice behaviour in the context of the specific challenges faced by the spider in its natural habitat.

a **b**

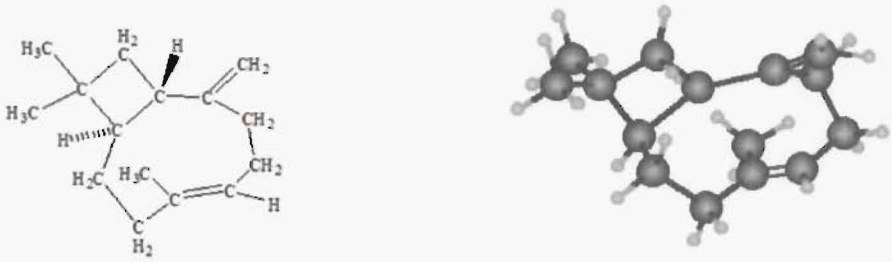

Figure 1. Structure of the sesquiterpene β-caryophyllene. **a)** In conventional chemical representation (figure courtesy of Andy Pratt). **b)** Three-dimensional image. From: http://www.launc.tased.edu.au/online/sciences/agsci/essoil/blarant.htm (accessed 16.11.04).

The shores of Lake Victoria, where *E. culicivora* makes its home, are rich with potential dipteran prey. The most numerous dipterans are non-biting midges in the families Chaoboridae and Chironomidae, known locally as 'lake flies' (Fig. 2). However, it is a less common dipteran, blood-fed female mosquitoes, that form the vast majority of *E. culicivora's* diet (Wesolowska & Jackson, 2003). In this chapter I wanted to investigate to what extent the adult spider is capable of identifying its preferred prey of mosquitoes in the presence of a surplus of lake flies and whether this prey-choice behaviour is modified by environmental chemical cues from *L. camara* and, in particular, by β-caryophyllene.

Figure 2. Lake fly (unknown sp.) on *Lantana camara*. Photo courtesy of Robert Jackson.

Materials and Methods

Purification of β-caryophyllene

The purification of β-caryophyllene was conducted in the Chemistry Department at the University of Canterbury, New Zealand. Because in previous experiments (Chapter 9) the available sample of β-caryophyllene was impure, a small amount was purified for these experiments. Purification of caryophyllene was achieved by flash chromatography with Merck silica 60 as the solid phase support. The eluting solvent was redistilled petroleum ether (b.p. 50-70°C). Purification was assessed using analytical thin layer chromatography conducted on aluminium-backed Merck Kieselgel KG60 F254 silica plates. Components were visualized using short wavelength UV light. Pure caryophyllene was isolated by evaporation of the petroleum ether solvent on a Büchi rotary evaporator and characterised by proton nuclear magnetic resonance spectroscopy (^{1}H NMR).

For NMR spectroscopy, β-caryophyllene samples were dissolved in deuterated chloroform ($CDCl_3$) and spectra were measured on the resulting solutions. The ^{1}H NMR spectroscopy of β-caryophyllene was performed on a Varian XL 300 instrument operating at 300 MHz. The probe temperature was 23°C. Chemical shifts, δ_H, were measured in parts per million on the δ scale.

Testing with virtual prey

The study was conducted at the Spider Laboratory at the University of Canterbury. All living spiders came from laboratory culture, and standard spider-laboratory procedures were adopted (Jackson & Hallas, 1986). All testing was carried out between 0700 h and 1200 h.

Methods for projection and the apparatus used for tests were identical to those used in the experiments described in Chapter 9, except that the virtual prey presented to the spiders were different and only adults of *E. culicivora*, that had been subjected to a 5-7 day pre-test fast, were tested. Test duration was as in previous studies (Chapter 4, 9).

Spiders were presented with side-on views of animated virtual mosquitoes and lake flies (for drawing and animation methods, see Appendix I). Virtual mosquitoes were derived from blood-fed females of *Anopheles* and lake flies were derived from *Chaoborus* sp. Both types of prey were animated to be grooming intermittently (for details, see Appendix I) and simultaneously. Virtual mosquitoes and lake flies were projected side-on so that potential cues from the head as well as from the abdomen were visible to spiders. All virtual prey measured 3.2 mm long on the focusing screen.

Unlike previous tests (Chapter 3, 4, 5, 7, 9), in which spiders were presented with two virtual prey, during these tests spiders were presented with four virtual prey, two at the bottom of the screen

and two at the top. Three of the virtual prey were identical lake flies and the other was a blood-fed female mosquito (Fig. 3). The position of the mosquito in these tests was not randomised. Instead it was always on the top right-hand corner of the screen. All virtual prey in this test were presented in greyscale (black and white).

Unsuccessful tests were those in which the spider walked down the ramp (away from the projected image), tests which lasted longer than 15 min without the spider stalking prey (see Chapter 10 for definition of stalking) and tests in which the spider jumped off the ramp or failed to move. If a test with a spider was unsuccessful, the same spider was sometimes used for same test on the same day (at least 60 min later), but spiders were never tested more than twice. The criterion for a successful test was seeing the spider jump, and land directly on, a virtual prey.

There were three tests in this series. In all tests, spiders were presented with the same selection of virtual prey in the chamber. In the first test, spiders were presented with virtual prey in the absence of odour cues. This served as a control with which the results from tests using odour were compared. In the second series of tests, the same virtual prey were presented to spiders but two inflorescences of *Lantana camara* flowers had been placed under the stainless steel platform 5 min before testing and for the duration of the experiment. In the third series of tests, spiders were again presented with the same virtual prey, but in this case the odour provided was β-caryophyllene.

Methods for depositing the 4 µl sample of β-caryophyllene, for placing the β-caryophyllene sample in the experimental chamber and details concerning the maintenance of odour in the experimental chamber and cleaning were identical to those described in Chapter 9. The only difference was that in these tests only β-caryophyllene was used.

As results from testing males were never statistically distinguishable from results from testing females of *E. culicivora*, data were pooled. Data were analysed using chi square tests of independence (Zar, 1984).

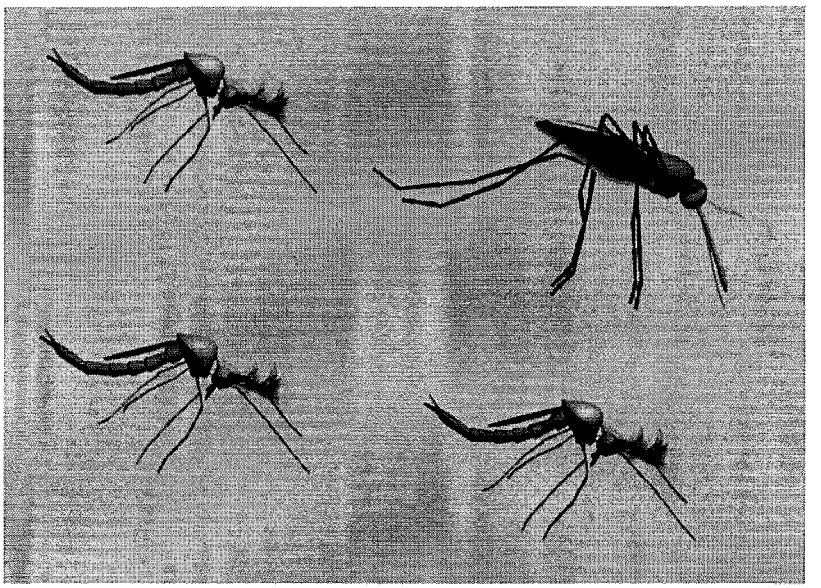

Figure 3. Image of the virtual prey scene presented to adult *Evarcha culicivora*. Top right: blood-fed female *Anopheles gambiæ* mosquito. Others: *Chaoborus* sp. (lake fly).

Results

Purification of β-caryophyllene

The ^{1}H NMR spectrum (Fig. 4) of purified β-caryophyllene was exactly in accordance with the distinctive NMR spectrum of β-caryophyllene reported in the literature (Hübner *et al.*, 1997), confirming the identity and high purity of the sample produced.

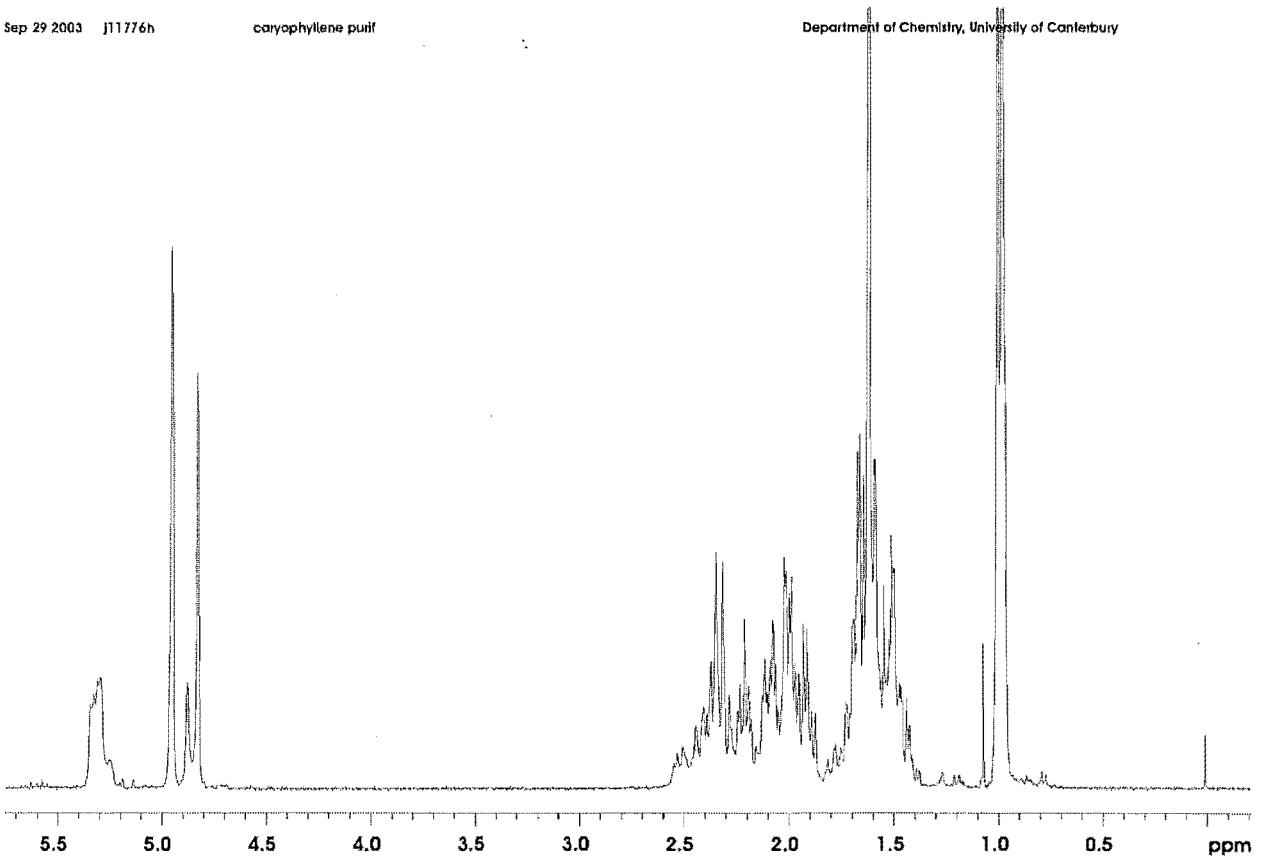

Figure 4. Proton nuclear magnetic resonance spectra of β-caryophyllene.

Testing with virtual prey

Chi-square tests of independence comparing the number of choices for mosquitoes and the number of choices for lake flies in each of the three treatments (no odour, odour of *L. camara* and vapour of β-caryophyllene), showed that the number of choices for mosquitoes and for lake flies with the odour of *L. camara* and with the odour of β-caryophyllene were not significantly different from each other (χ^2=0.01, NS, N=39) (Fig. 5). However, when comparisons were made between the choices in the no odour treatment and with the odour of *L. camara*, there was a significant difference in *E culicivora's* prey-choice behaviour between treatments (χ^2=5.55, P<0.02, N=38) (Fig. 5), i.e., *E. culicivora* chose mosquitoes more often in the absence of odour than in the presence of odour of *L. camara*. When comparisons were made between the choices made in the no odour treatment and with the vapour of β-caryophyllene, there was a significant difference in *E culicivora's* prey-choice behaviour (χ^2=5.53, P<0.02, N=41) (Fig. 5), i.e., *E. culicivora* chose mosquitoes more often in the absence of odour than in the presence of β-caryophyllene.

There was no evidence that the presence of odour affected the number of successful tests. The number of failed tests with and without odour were not significantly different to each other. In the absence of odour, there were 20 successful and 24 unsuccessful tests. In the presence of odour from *L. camara*, there were 18 successful and 15 unsuccessful tests (χ^2=0.623, NS, N=77) and in the presence of odour from β-caryophyllene, there were 21 successful and 16 unsuccessful tests (χ^2=1.02, NS, N=81).

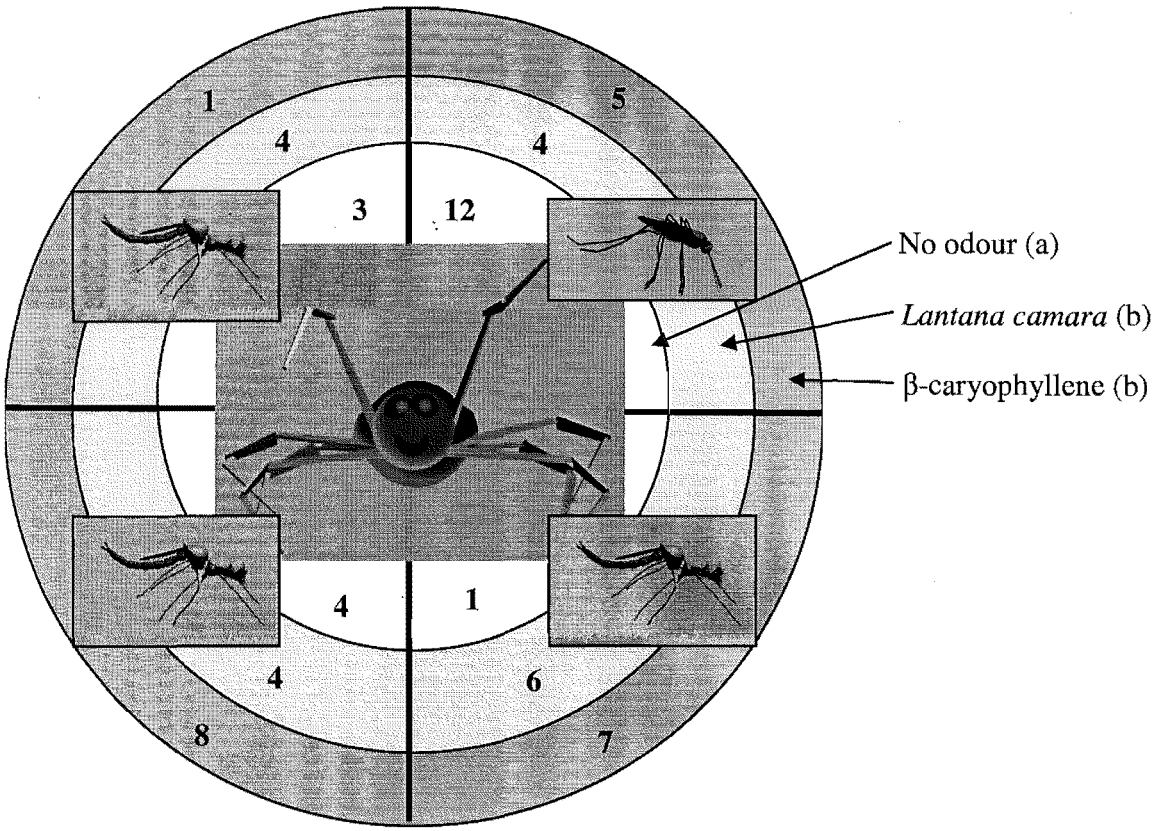

Figure 5. Results from vision-based tests with adult *Evarcha culicivora* in three treatments: no odour, odour from *Lantana camara* flowers, odour of β-caryophyllene. Spiders simultaneously shown four virtual prey: three 'lake flies' and one blood-fed female *Anopheles* mosquito. Inner ring shows numbers that went toward each choice (inset figures) in the absence of odour cues. Middle ring: in the presence of the odour from *Lantana camara* flowers. Outer ring: in the presence of β-caryophyllene. Chi-square tests of independence (null hypothesis: choose each prey type equally often regardless of treatment). Treatments having significant differences ($P<0.02$) denoted by different letters in parentheses beside the treatment description on the right-hand side of the diagram.

Discussion

The results presented in this chapter provide strong support for the notion that *E. culicivora* no longer chooses its preferred prey of mosquitoes (Chapter 3) in the presence of the odour of *Lantana camara* any more than would be expected by chance (i.e., there was no obvious choice behaviour). These results further suggest that β-caryophyllene is a proximate cause in the exhibited change in behaviour of *E. culicivora*. This is the first report of a spider's prey-choice behaviour being modified by a single environmental chemical cue (β-caryophyllene) not pertinent to the prey.

Without the odour of *L. camara* flowers or of β-caryophyllene, *E. culicivora* managed the cognitively demanding task of locating its preferred prey of mosquitoes in the presence of a surplus of lake flies. The mosquito was nestled among three identical midges of similar appearance to the mosquito. This resembles the challenge faced by *E. culicivora* in nature, because mosquitoes are vastly outnumbered by the similar-looking lake flies in *E. culicivora's* habitat (pers. obs.). Results from previous studies indicated that *E. culicivora* can distinguish between midges and mosquitoes using solely visual cues (Chapter 3). However, this is the first experimental evidence that, in the presence of multiple similar-looking prey, *E. culicivora* can identify the single mosquito based solely on visual cues.

Interestingly, in the presence of both *L. camara* flowers and β-caryophyllene *E. culicivora's* behaviour changes in a very unusual way; it no longer chooses mosquitoes in preference to other prey (i.e., the spider's prey-choice appears to be random). This behavioural change is significant because *E. culicivora* is attracted to *L. camara* (Chapter 8) and is often found on these plants in nature. β-Caryophyllene is found in a wide variety of flowering plants, not just *L. camara* (da Silva *et al.*, 1999; Weissbecker *et al.*, 2000; Khan *et al.*, 2002; Araujo *et al.*, 2003), and is known to attract several insects (Weissbecker *et al.*, 1999; Zhu *et al.*, 1999; Al Abassi *et al.*, 2000; Kalinová *et al.*, 2000; van Loon *et al.*, 2000; Hammack, 2001; Bichão *et al.*, 2003; Syed & Guerin, 2004) but has never been tested for its effect on spiders. Although the nature of *E. culicivora's* attraction to *L. camara* (Chapter 8) remains unknown, tests are underway to determine if β-caryophyllene is attractive to *E. culicivora* (RRJ, pers. comm.).

This behavioural change is somewhat paradoxical, given that *E. culicivora's* prey preferences are no longer expressed on *L. camara* but that *E. culicivora* is attracted to it. As suggested in Chapter 9, the benefits of finding large numbers of prey congregating on *L. camara*, especially mosquitoes, which are few and far between compared with lake flies, are likely to outweigh any deleterious effect of β-caryophyllene on *E culicivora's* behaviour.

To determine whether motivation to attack was affected by odour, I compared the number of spiders that responded to tests with the number of spiders that did not respond, for the three treatments (no odour, *L. camara* and β-caryophyllene). Overall, there were high numbers of spiders that did not respond in these tests (about 50%), but this probably reflects the cognitively demanding task the spiders were faced with, as well as the stringent criteria for what constituted 'choice'. The results from comparing the 'no response' values with the 'response' values were not significantly different between treatments. However, there was a slightly higher response toward lures in the presence of odour (from both *L. camara* and β-caryophyllene) than when absent. These results do not suggest that odour impaired *E. culicivora's* motivation to attack (acting as a depressant); if anything, odour appeared to increase the spider's motivation to attack (acting as a stimulant).

It seems entirely plausible that the change in *E. culicivora's* prey-choice behaviour is a case of the spider going 'mad' (or being on a 'trip') and that the drug that induces the trip is β-caryophyllene. However, as long as the spider's motivation to attack is not impaired, the benefits of finding an area with large numbers of prey (including their preferred prey) probably outweigh any costs of killing non-blood-carrying prey. The benefits for *E. culicivora* of being in the presence of *Lantana* may extend further than simply finding a surplus of food. There is the possibility that, by preying indiscriminately, *E. culicivora* obtains a mixed diet, which may be more advantageous (Evans *et al.*, 1999) to the spider than a pure diet of blood-fed female mosquitoes. In fact, evidence to date supports the notion that a mixed diet is better for *E. culicivora* than a pure diet of blood-fed female mosquitoes (RRJ, unpublished data).

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CHAPTER ELEVEN

Discussion

A spider's-eye view of a mosquito

"The Brahmins assert, that the world arose from an infinite spider, who spun this whole complicated mass from its bowels, and annihilates afterwards the whole or any part of it, by absorbing it again, and resolving it into his own essence. Here is a species of cosmogony, which appears to us ridiculous; because a spider is a little contemptible animal, whose operation we are never likely to take for a model of the whole universe. But still here is a new species of analogy, even in our globe. And were there a planet wholly inhabited by spiders (which is very possible), this inference would there appear as natural and irrefragable as that which in our planet ascribes the origin of all things to design and intelligence, as explained by Cleanthes. Why an orderly system may not be spun from the belly as well as from the brain, it will be difficult to give him a satisfactory reason." (Pt. VII.)[1]

Although this thesis is *not* in the business of trying to discover the "origin of all things to design and intelligence", the Brahmin spider need not be the object of ridicule in which design is spun by the spider's belly. Perhaps now, 200 years later, the spider's brain may be considered as a viable system for understanding the ultimate 'design': intelligence.

The very notion of intelligence among spiders is one that most people balk at, but more and more studies in cognitive ethology and behavioural ecology are beginning to address cognitive issues, not only in mammals, but in insects and in spiders (Srinivassan *et al.* 1998; 1999; Srinivassan & Zhang, 1998; Laloi *et al.*, 1999; Menzel & Guirfa, 2001; Wilcox & Jackson, 2002; Hebets, 2003; Greenspan & van Swinderen, 2004; Harland & Jackson, 2004). Until recently, cognition in non-human animals was simply a black box that did not need to be addressed (Giraldaeu, 2004). But it is now becoming clear in biology that *essence*, in the philosophical sense, has no binding margins. There cannot be a fundamental distinction of what constitutes the 'intelligence' and 'non-intelligence'. The ability to flexibly respond to problems in some (particularly problematic) habitats is a "Good Trick" (*sensu* Dennett; 1995), and, since "minds are what brains do" (Minsky, 1986),

[1] Hume, D. 1779. *Dialogues concerning natural religion.* London. Cited in: Dennett, D. C. 1995. *Darwin's dangerous idea.* London: Allen Lane. Pp. 31-32.

intelligence is likely depend on the needs of each animal within its particular niche. It may be best to think of intelligent behaviour as 'stepping stones' interspersed throughout all animals.

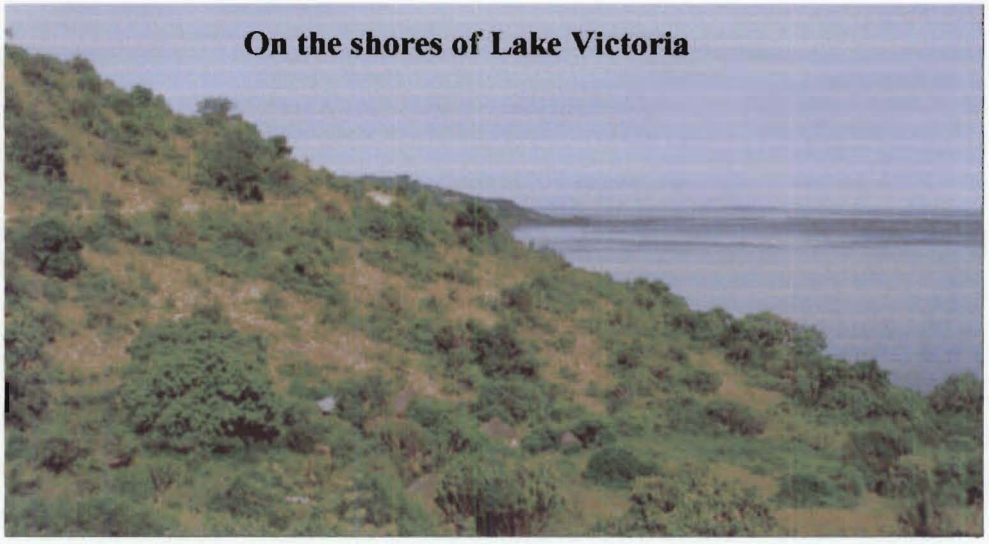
Compared with birds and mammals, most arthropods have minute nervous systems and this may appear to impose drastic limitations on their potential cognitive capacity. Nevertheless, there is considerable evidence that spiders display significant cognitive abilities. Furthermore, having small nervous systems, they are useful subjects for research on learning (e.g., Morse 2000; Nakamura & Yamashita, 2000; Seah and Li 2001), problem solving (e.g., Jackson and Wilcox 1994; Tarsitano & Andrew, 1999; Jackson and Carter 2001; Jackson *et al.*, 2001a) and other topics related to animal cognition (Wilcox and Jackson 1998, 2002; Harland & Jackson, 2004; Jackson & Li, 2004).

Among jumping spiders (Salticidae), species with unusual prey-choice behaviour appear to be especially useful for animal-cognition research (Richman & Jackson, 1992; Jackson & Pollard, 1996) and, by choosing blood-fed female mosquitoes as their preferred prey, the East African salticid *Evarcha culicivora* has the most unusual prey-choice behaviour of all salticids described to date. The central question for the work presented in this thesis was 'how does *E. culicivora* find mosquitoes amid all the 'noise' in its habitat, especially amid all the non-biting midges (known locally as 'lake flies') there?'

This thesis was divided into two sections following from an introduction to the thesis (Chapter 1) and to malaria (Chapter 2). In the first section (Chapters 3-7) ("Prey preferences of *Evarcha culicivora*"), I addressed the prey-choice decisions made by *E. culicivora* using two different sensory modalities, olfaction and vision. I also investigated the visual features of mosquitoes that are used by *E. culicivora* to identify prey. In the second section (Chapters 8-10) ("Plant-odour effects on *Evarcha culicivora's* prey-choice behaviour"), I investigated how features of *E. culicivora's* environment may affect its behaviour. In particular, I investigated the effect on *E. culicivora's* prey-choice behaviour of volatiles from *Lantana camara*, a plant to which *E. culicivora* is attracted.

The fact that *E. culicivora's* habitat, near Africa's Lake Victoria, is a region of endemic malaria, and the fact that *E. culicivora* selects mosquitoes as its preferred prey, may have important consequences for its potential use in the biological control of the vectors of this disease. Furthermore, that *E. culicivora* can discriminate between mosquitoes and other very similar looking dipterans (lake flies) has implications concerning the visual-cognition abilities of salticids.

Interesting questions are also raised regarding how the biology of a spider may have become linked to a particular plant species, *Lantana camara*.



The shores of Lake Victoria, the source of the White Nile and Africa's largest lake, teem with invertebrate life, from army ants to dung beetles, but 'lake flies' (chironomid and chaoborid dipterans) seem to swamp everything else. *Evarcha culicivora's* preferred prey, blood-carrying mosquitoes, are only a tiny minority in a sea of lake flies. In this habitat *E. culicivora* finds and eats mosquitoes, as its name implies. Sometimes, swarms numbering in the billions of lake flies get blown across the lake (Fig. 1a) to shore (Carpenter, 1920; Beadle, 1981). When these insect storms hit the shore, they cover almost everything (Fig. 1b,c,d). This highlights the central theme of this thesis: how does *E. culicivora* find the mosquitoes amid all the 'noise' of lake flies.

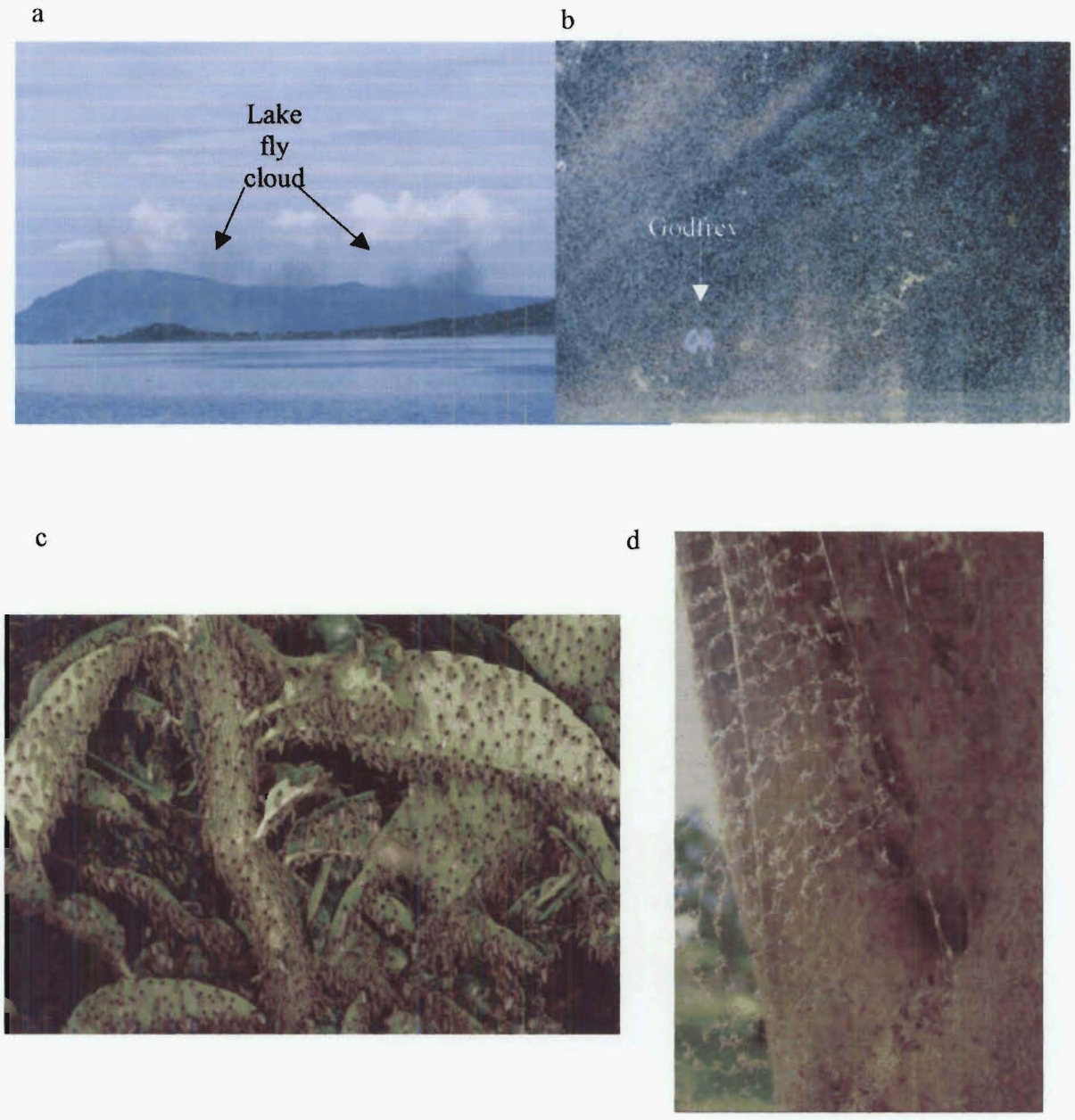
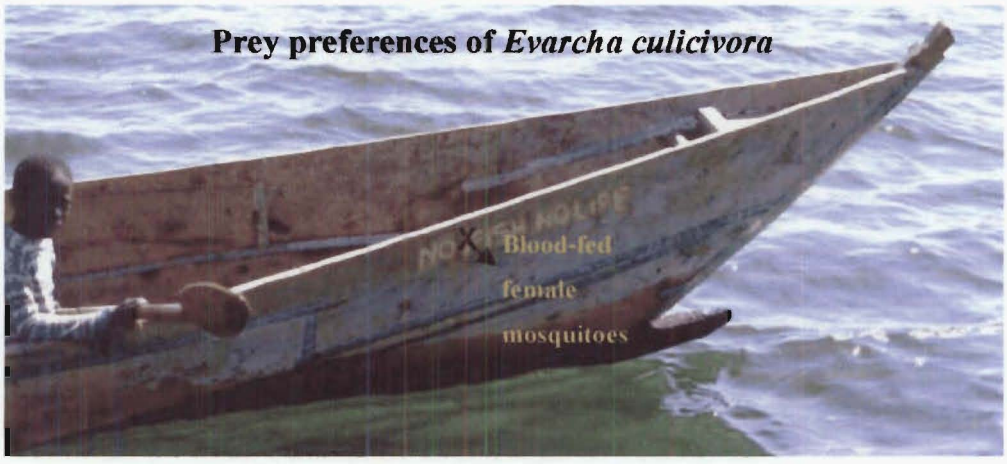


Figure 1. Lake flies on the shores of Lake Victoria. **a)** Lake-fly cloud, composed of billions of midges, being blown to shore. **b)** Lake-fly cloud after reaching the shore at Mbita Point in Kenya (Godfrey Sune, a member of the spider-research team, is almost hidden by the fly storm). **c)** Close-up of lemon-tree with lake flies (each dark spot is the head of an individual lake fly). **d)** Lake flies, the most numerous potential prey for spiders, swamping the web of *Nephilengys* sp. at Mbita Point, Kenya. Photos **a)** and **b)** courtesy of Robert Jackson.



- By eyesight alone, *Evarcha culicivora* is efficient at identifying female mosquitoes that have recently fed on blood, discriminating them from a wide range of other potential prey, including male mosquitoes, female mosquitoes that have not fed on blood, various species of lake flies, fruit flies, aphids and spiders (Chapter 3).

The eyes of most spiders lack the structural complexity required for acute vision (Homann, 1971; Land & Nilsson, 2002), but the unique complex eyes of salticids support resolution ability with no known parallel in other animals of comparable size (Land 1969a, b; Williams and McIntyre, 1980; Blest *et al.*, 1990). Salticids have four pairs of eyes. Three pairs, called the 'secondary eyes', are positioned around the sides of the cephalothorax and function primarily as motion detectors (Crane, 1949; Land, 1971; Dill, 1975; Forster, 1985). However, it is the large pair of forward-facing eyes, called the 'principal', or 'anterior-median' (AM), eyes, that are responsible for acute vision (Land, 1969a,b; Williams & McIntyre, 1980; Land & Nilsson, 2002). Although large for a spider, the salticid's AM eyes are very small by vertebrate standards (see Appendix II) and they are structured very differently (Land, 1985).

The retina of the principal eye has a four-layer tiered arrangement, and light entering the corneal lens passes successively through layers IV, III and II before reaching layer I. The central area of layer I, the fovea, is the only region that receives a sharply focused image (Blest *et al.*, 1981, 1990). Because of the small amount of space in the salticid's body, there are only a few hundred receptors within the fovea (Land, 1974; Blest *et al.*, 1990). An eye with so few receptors cannot be operating on the same principles as the larger eyes of vertebrates (Land, 1985). Yet this tiny eye

(Appendix II) somehow resolves details of shape and form sufficiently to distinguish female mosquitoes from other dipterans of the same size, such as lake flies, and even from conspecific males (Chapter 3). One way in which the fovea is thought to compensate for its diminutive size is by movement. Through a specialised arrangement of muscles (Land, 1969a), the salticid is able to 'sweep' the minute fovea, located at the end of an eye-tube, across the much larger image of its target. Taking into account the eye movements of the principal eyes, the visual field of salticids extends to about 35° on either side of the body axis (Land, 1972), and so offsets the limitations of the fovea's narrow field of view. We know of four different types of movement that the salticid eye can perform (Land, 1969a,b; 1985), of which 'scanning' is probably the most important because it appears to have a primary role in target identification. When scanning, the eye-tube undertakes elaborate movement routines in which they rotate and move side-to-side. The scanning behaviour of the salticid's eye-tubes may be a model system for studying feature-detector systems with similarities to what we know about how frog eyes identify prey (Barlow, 1953a,b; 1982; 1996; but see Roth, 1986). For the frog, much of the decision-making underlying prey-identification takes place in the eyes instead of the central nervous system, and perhaps much of a salticid's decision-making is made by the AM eyes. However, if we take the literature at face value, there are striking differences in how information matters to the salticid compared with the frog. For the frog, it seems that prey is simply something fly-size that is moving, whereas salticids seem to routinely be paying attention to more fine-grain features (reviewed in Harland & Jackson, 2004).

Myrmecomorphic, or ant-like, salticids provide some interesting examples. These salticids live in the vicinity of ants but the myrmecomorphic salticid preys primarily on safer prey. Ants are, for the myrmecomorphic salticid, potential predators (Nelson *et al.*, 2004; Nelson *et al.*, in press). Until we become experienced at identifying them, ants and myrmecomorphic salticids are almost indistinguishable both morphologically and behaviourally (Cushing, 1997). However, in the absence of tactile or chemical cues, myrmecomorphic salticids routinely distinguish other myrmecomorphic salticids from ants (Nelson, 1998), albeit (and interestingly) at shorter distances than other salticids distinguish between conspecifics and prey (Harland *et al.*, 1999). The experimental work (Chapter 4) on prey identification by *Evarcha culicivora* is another illustration of a salticid attending to fine-grain details of the targets.

- The specific optical cues on which *Evarcha culicivora* relies for prey identification appears to include the outlines of the antennae, the shape of the abdomen, the behaviour of the prey

and the prey's size. Over all, *E. culicivora* most often chooses female mosquitoes and specifically females that have fed on blood and are moving in a natural manner. The characteristically engorged shape of the abdomen of blood-fed mosquitoes appears to be an important identification cue (Chapter 3, 4). Cues from the antennae appear to be attended to when the mosquitoes in view have not had blood meals (i.e., as a cue, the shape of the mosquito's abdomen appears to take precedence) (Chapter 4). Prey size also influences prey-choice behaviour (Chapter 3, 4): larger size-classes of the spiders choose larger mosquitoes (as long as they have fed on blood, i.e., blood is the overriding factor in prey-choice decisions), but the smaller size classes of *E. culicivora* choose smaller mosquitoes (also as long as they are blood-engorged).

The results from Chapters 3 and 4 strongly suggest that *Evarcha culicivora* 'looks for' certain key features from the prey (e.g., engorged mosquito means blood). Along with *Portia*, an araneophagic (spider-eating) salticid, *E. culicivora* may be a model animal for research on how complex prey-choice behaviour and acute vision are tightly interrelated. Salticids may have evolved mechanisms that keep the amount of information processing low enough for a small nervous system to handle. For example, *Portia fimbriata* adopts a specialised gait when stalking other salticids ('cryptic stalking'; Jackson & Blest, 1982), but not when stalking ordinary spiders or insects. A primary salticid-identification cue used by *Portia* is the shape and size (relative to prey body width) of the prey's anterior-median eyes (Harland & Jackson, 2000; 2002). Legs, and, to a lesser extent, cues from the abdomen are secondary cues for salticid identification (Harland & Jackson, 2000; 2002). Harland and Jackson (2000) suggested that, instead of an algorithm expressed in terms of a series of if-then statements, *Portia* may be using an algorithm based on a set of independent perceptual processes, each with the task of identifying a specific cue, and a set of response processes, each mediating different predatory tactics. When a perceptual process identifies a relevant cue, it might activate one or more response processes: i) a general predatory response (to stalk or not to stalk) that is activated when the perceptual processes identify some combination of leg based, anterior-median eye-based and abdomen-based cues (in that order of importance); ii) a more specific predatory response that is readied when the perceptual processes identify anterior-median eye-based cues (activated only when the predatory response is activated). An algorithm of this type would maintain neural economy while supporting considerable flexibility and apparent complexity.

- Compared with the larger juveniles and adults of *Evarcha culicivora*, the smaller juveniles of *E. culicivora* make especially specific prey-preference decisions: they choose mosquitoes in the genus *Anopheles* in preference to other mosquito genera. The primary *Anopheles*-identification cue appears to be the characteristic resting posture of *Anopheles*. This is the first report of any predator selecting *Anopheles*, the vectors of human malaria, as preferred prey (Chapter 5). Furthermore, the smaller juveniles of *E. culicivora* have an *Anopheles*-specific prey-capture tactic that appears to be an especially effective method by which the minute predators (body length *c.* 1.5 mm) can subdue the mosquito (Chapter 6).

Anopheles gambiæ is the principal malaria vector in Sub-Saharan Africa. The females of *An. gambiæ* (Constantini *et al.*, 1998) require blood meals to complete their gontotrophic cycle and lay eggs, and they are strongly anthropophilic (i.e., they especially feed on human blood). *An. gambiæ's* effectiveness as a vector of malaria is strongly enhanced by the unusual longevity of the females of this species. However, various environmental factors influence the transmission of malaria by *An. gambiæ*. Depending on temperature, the incubation period of *Plasmodium* (the malaria parasite) in the mosquito midgut takes between 10 and 20 days. In continual warm climates, such as that found in Mbita Point on Lake Victoria, *Plasmodium* completes this stage of its life cycle in closer to 10 days than to 20 days. The exceptional longevity of *An. gambiæ* combined with the rapid development of *Plasmodium* in warm climates combine to make biting frequency the more important factor for malaria transmission in equatorial Africa compared with colder climates where vector longevity is the more important factor (Gillot, 1980; Clements, 1999; http//www.cdc.gov/malaria/biology/index.htm, accessed 02/09.04).

It is widely accepted that the only way that is currently realistic to control malaria in Africa is by interrupting the transmission of the *Plasmodium* parasite (Miller & Greenwood, 2002). If, on average, each infected person transmits malaria to fewer than one other person, the *Plasmodium* population will not be sustained. *Evarcha culicivora*, by selecting *Anopheles* females as prey, may have some significance in breaking the cycle of transmission, but it is premature to predict how useful it might be. However, educating people to recognize this spider, and not to kill it in their homes, might be advisable.

Regardless of potential applied significance, the *Anopheles*-specific predatory behaviour of the smaller juveniles of *E. culicivora* (Chapter 6) appears to be especially suitable for further investigations of the type of interactive algorithm proposed by Harland & Jackson (2000, 2002). For

juveniles, the algorithm might look like this: i) a general predatory response ('to stalk or not to stalk') that is activated when the perceptual processes identify some combination of antennae-based, abdomen-based, and possibly colour-based cues; ii) a more specific predatory response that is readied when the perceptual processes identify posture-based cues and is activated only when the predatory response is activated. However, an algorithm of this type has a drawback. It can only be studied in predators that have prey-specific predatory behaviour.

To understand how salticid eyes achieve prey identification while operating within the neural constraints imposed by the spider's size, the feature-detector model (Barlow, 1953 a,b; 1996) may be the best approximation we have. Even less is known about the seemingly harder question of how salticids overcome size constraints on brain function and cognition. For example, if the salticid is making its decisions based on features that it extracts from its view of the target, this implies that the 'bits' that have been scanned and found to have the correct features must be kept in working memory (see Miller, 1956; Dukas, 2004) so that it can all be added-up in the end to make a comprehensive whole on which to base a decision. Even if dealing with only simple lines, there are several of them and it appears that the salticid would need to remember the relative spacing, size and angle of each of them.

- Visual prey identification by *Evarcha culicivora* appears to be more complex than normally expected for arthropods, with multiple factors interacting to influence prey-capture decisions. The possibility that colour is important was considered in Chapter 7. The colour red (long wavelength light) seeming to be a cue that tells *E. culicivora* that the mosquito it is attacking has fed recently on blood (see Fig. 2). A series of questions that *E. culicivora* might ask itself when making prey-choice decisions based on vision, are summarised in Figure 2.

Based on morphology and physiology, that the anterior median (AM) eyes of salticids can support colour vision is widely accepted (Land, 1969a,b; De Voe, 1975, Yamashita & Tateda, 1976; Blest *et al.*, 1981; Blest *et al.*, 1990), despite little being known about how colour actually influences salticid behaviour. Attempts to determine the precise spectral sensitivities of the AM eyes of salticids have yielded mixed results. Some authors have suggested that salticids can detect long-wavelength light (red) (Land, 1969a; Peaslee & Wilson, 1989), whereas others, studying different species, have suggested that salticids do not detect red (De Voe, 1975; Yamashita & Tateda, 1976; Blest *et al.*,

1981). Perhaps a consensus has not been reached in part because there is considerable variation in the spectral sensitivities of the eyes of different species of salticids. One possible explanation for this variation is that the eyes are well-adapted to the local niche-driven needs of each species.

Mosquitoes that have recently fed on blood become engorged and typically their abdomens take on a distinct red appearance which diminishes in brightness with time. If salticid colour vision is niche-driven, ability to detect red may be especially important for *Evarcha culicivora* because this is a predator that singles out blood-filled prey. If substantiated, this finding will be the first confirmation of the use of colour in the decision-making processes of a salticid.

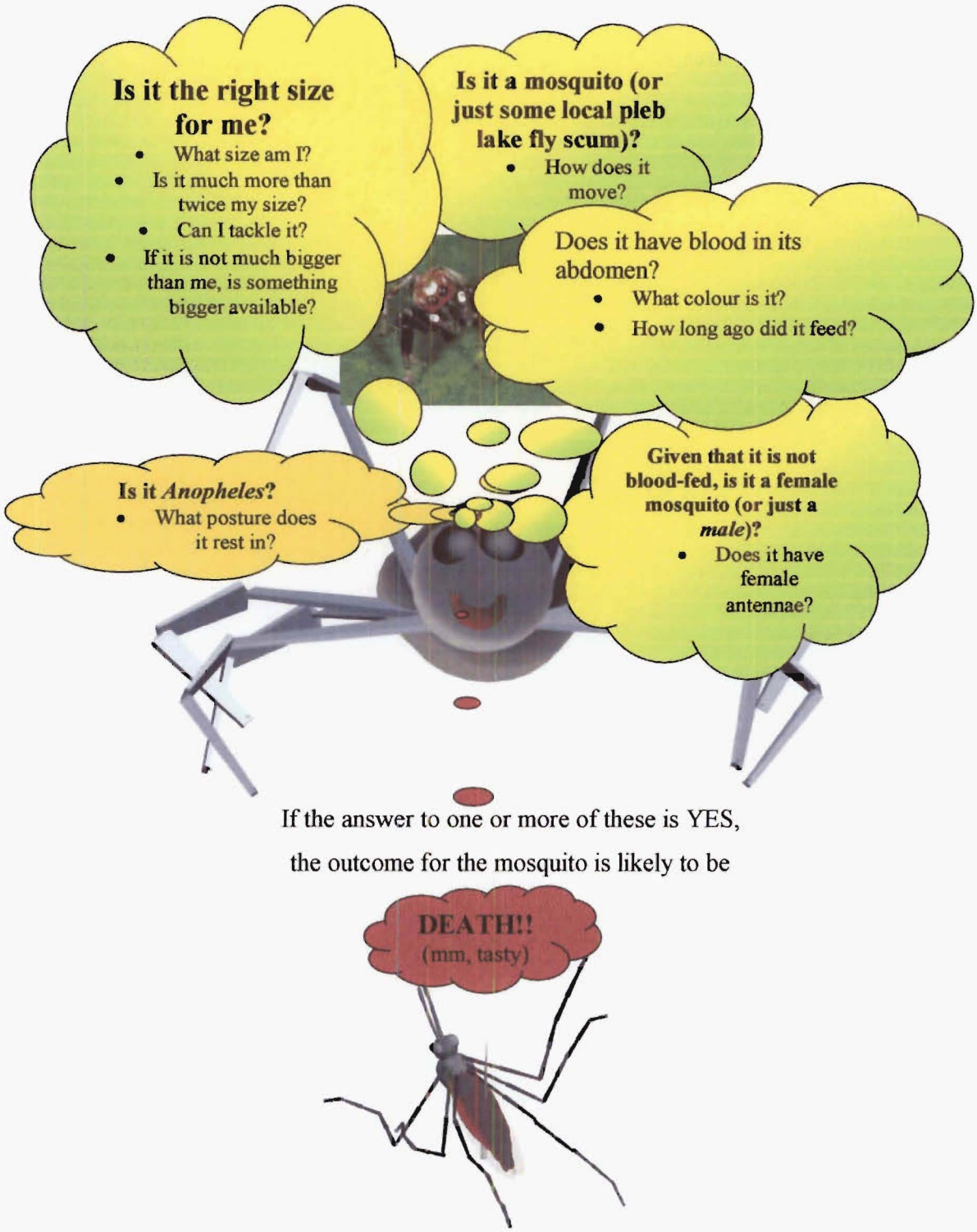


Figure 2. Decision-making processes of *Evarcha culicivora*. For heuristic purposes, a non-literal portrayal is presented here of the questions that *E. culicivora* may ask before stalking its prey. Approximate order of importance suggested by the font size (bigger: more primary). Orange and red are separate 'thought-processes'.

Obtaining experimental evidence of the role of colour in salticid behaviour is hindered by the inherent difficulties of manipulating colour on real animals. Although seldom used (but see McKinnon & McPhail, 1996; Harland & Jackson, 2002), animation techniques may provide an effective method (Rosenthal, 2000) for exploring the controversial issue of colour vision in non-human animals (see Cuthill *et al.*, 2000). My work with *E. culicivora* suggests that the potential problems with the use of animation can be overcome in studies using salticids.

Playback techniques have been regarded as potentially powerful tools in experimental studies of animal behaviour because, with these techniques, single variables can be modified in a controlled manner and in a single sensory modality. Video playback has been used successfully with salticid and lycosid spiders (Clark & Uetz, 1990; Persons & Uetz, 1997), fish (McKinnon, 1995) and birds (Evans & Marler, 1992). However, there have also been several instances (mostly unpublished, see: Schlupp, 2000) of unsuccessful attempts to use video playback (Gonçalves *et al.*, 2000; reviewed in Schlupp, 2000).

Critics of the use of video playback have been concerned primarily with problems associated with depth perception (Zeil, 2000), colour vision and visual acuity of animals with different spatial resolution from our own (D'Eath 1998; Fleishman *et al.*, 1998, Fleishman & Endler, 2000). I discuss each of these problems with respect to the methods and findings from this thesis.

The ability to perceive depth cues on a two-dimensional plane may be a significant problem. I attempted to reduce the problem of depth perception by creating animations on a non-textured grey background, and I appear to have been at least somewhat successful. Video footage of *E. culicivora* leaping on its choice of virtual prey shows that the spider leapt on the prey in a parabola, presumably intending to land on its dorsal surface (Fig. 3). That depth perception apparently was not problematic in these experiments was further corroborated by the fact that, when spiders leapt on their prey, they did so from the same distance that they would to real prey (*c.* 2 cm in adults of *E. culicivora*; pers. obs.) and that, in the hundreds of tests using animation I did during this thesis, less than 1% of the spiders that leapt on virtual prey did not leap far enough (i.e., misjudged the distance). Of course, if the spiders were aiming further away (i.e., 'beyond' the screen on which prey were projected), I would not detect it because the spiders still would have landed on the screen. However, it was very unusual for spiders not to land directly on their prey, and, if they were aiming beyond the screen, the chance that the spiders would have landed on the screen on top of the prey, rather than, say, above it- if jumping in a parabola (Fig. 3) - seems unlikely.

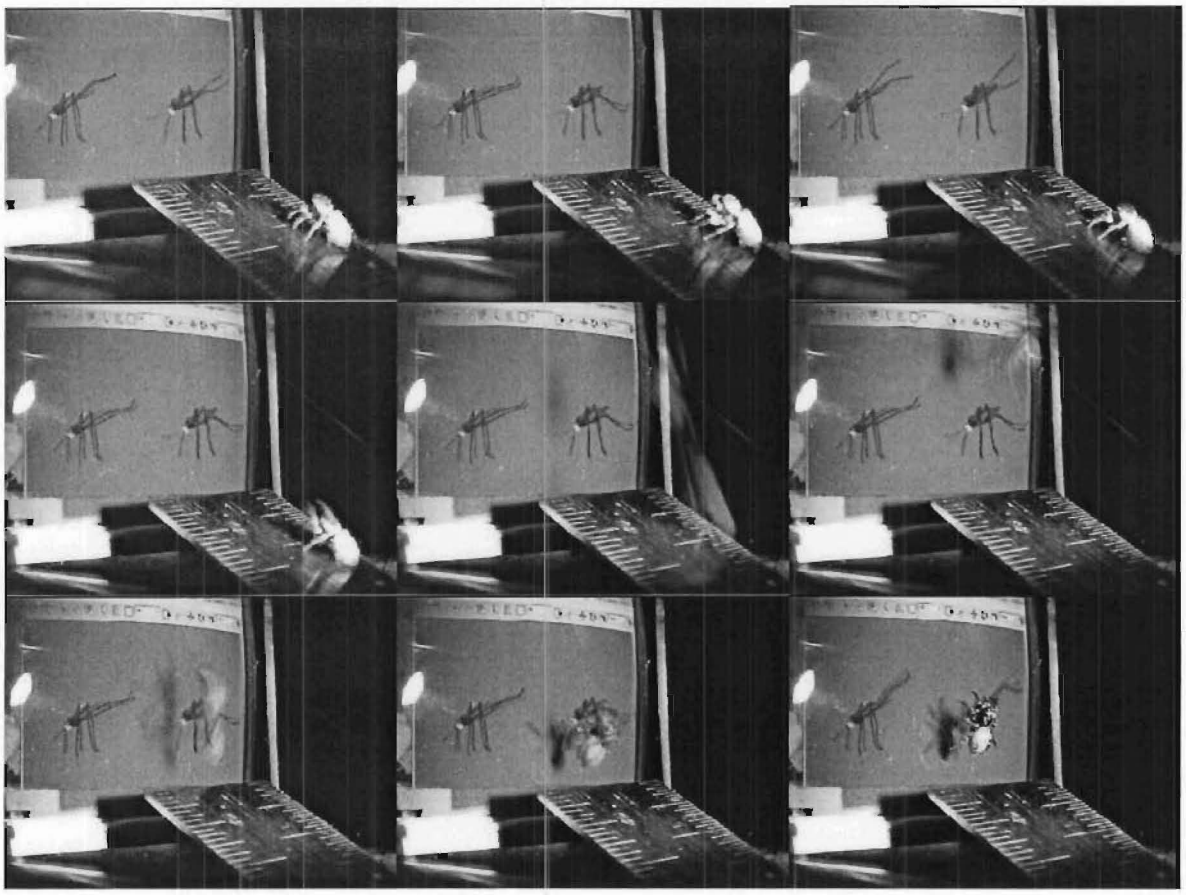


Figure 3. Sequential frames from a digital video sequence of adult *Evarcha culicivora* leaping on the virtual prey during preliminary experiments. Depth perception by *E. culicivora* indicated by spider's parabolic leap (seen in the shadow of the spider cast on the screen), which would have landed on dorsal surface of a real mosquito. Note: spider looks at both virtual prey (first three frames: right, left, right) before leaping. Note: this is typical predatory behaviour of adult *E. culicivora*.

Instruments for projection of images (projectors, television, etc.) are designed to simulate the colour of objects as humans see them rather than to faithfully reproduce the spectrum of light that they naturally emit, transmit, or reflect. This simulation is achieved by using relatively narrow waveband light to stimulate the cone cells in the retina in a similar pattern to that produced by the natural object. However, other animals differ in the number and spectral tuning of their photoreceptors (Fleishman & Endler, 2000). Furthermore, the perception of colour combines a transformation of the summed 'data' from the stimulation of the relevant photoreceptors in addition to a neural measure of the difference in stimulation of each of the different photoreceptor types (this

gives rise to hues), so faithful colour rendition for a human may not easily be achieved for another species (Cuthill *et al.*, 2000).

Although there are many problems associated with presentation of coloured stimuli to animals other than ourselves on purpose-designed machines used for human visual systems (data projectors, computer monitors and TVs, for example, do not have a UV component), surely this does not mean that we should shy away from studying the important question of colour perception in non-human animals? Particularly among animals with a strong visual component to their behaviour it is important to elucidate the effect of colour vision if we are to understand their behaviour in any kind of holistic manner. There are problems, undoubtedly, in ascribing the colours we see to the colours other animals may or may not see. However, spectral analysis of reflected wavelength distributions of the stimuli, coupled with comparative studies in which responses toward live animals and virtual animals are evaluated, provide a reasonable basis from which to start. Furthermore, comparative studies between virtual stimuli in which colours (wavelength distributions) are systematically varied could potentially provide valuable information about the colour discrimination of non-human animals.

Used in the manner described in this thesis with the jumping spider *Evarcha culicivora*, animation techniques offer all of the advantages of video playback while removing many of the disadvantages. Projecting the image with a data projector and through reducing lenses instead of presenting it to the study animal directly on a TV screen removes problems associated with the different levels of spatial acuity of other animals. Video images and images on a TV or on a computer monitor are made of thousands of tiny pixels. Animals with higher resolution than our own (e.g., raptors) may see an image in pixelated form. Salticids have excellent visual acuity (Blest *et al.*, 1990; Land & Nilsson, 2002), but not higher than our own. Furthermore, it is highly unlikely that a salticid would be able to differentiate between the pixels making up the mosquito when it is presented on a fine-grained glass screen, not on a monitor (and furthermore was reduced, with the aid of a reducing lens, from approximately 10 cm in length to 3.2 mm).

The frequency at which a flickering stimulus starts to appear continuous is known as the critical flicker fusion frequency. The problem of critical flicker fusion threshold, touted in the original literature (D'Eath, 1998), is erroneous. It has been claimed that, in order to create apparent smooth motion, the presentation rate of the video exceed the critical flicker fusion frequency of the animal, but this is not the case; the illusion of smooth motion in humans can easily be created well below the critical flicker-fusion frequency of approximately 50 Hz (Fleishman & Endler, 2000).

Nevertheless, even if the critical flicker fusion rate were a problem, in the methods used in this thesis I ensured that the computer monitor's refresh rate was 55 Hz during all tests, on the assumption that the critical flicker fusion frequency of salticids is 40 Hz (Forster, 1985). This was an unnecessary precaution because the stimulus was not presented to the spider on a monitor, but through a projector with a continuous light source.

The fact that *E. culicivora* responds at all to virtual mosquitoes is interesting, but that it actively stalks virtual mosquitoes and leaps on them in a manner identical to the way it would on real prey (pers. obs.; Fig. 3) strongly suggests that this technique is effective for the study of *E. culicivora's* behaviour and corresponds to the criterion of 'successful' use of playback techniques (D'Eath, 1998; Fleishman & Endler, 2000).

- By olfaction alone, *Evarcha culicivora* is efficient at identifying blood sources (female mosquitoes that have recently fed on blood) (Chapter 3).

Redundancy of sensory systems may be important for *Evarcha culicivora*. When deprived of one system, *E. culicivora* may be able to locate prey relying on another sensory modality. However, *E. culicivora* may often use vision and olfaction together. For a salticid that has acute eyesight, but lives in a complex 3D habitat, detecting prey at a distance may be difficult using vision alone. For initially detecting the presence of prey, olfaction may often be more effective than vision (see Persons & Rypstra, 2000; Uetz & Roberts, 2002). Perhaps, also, the odour of blood-fed female mosquitoes 'alerts' or 'primes' *E. culicivora* to the presence of a blood-meal so it can more readily identify the mosquito by sight (i.e., it may become prepared to see a mosquito). There have been only a few studies of cross modality priming between odour and vision by non-human animals (see Blough, 1989; Blough & Blough, 1997; Johnen *et al.*, 2000; Martin-Malivel & Fagot, 2001), with recent studies on araneophagic salticids being among the most detailed of these (Clark *et al.*, 2000; Harland & Jackson, 2001; Jackson *et al.*, 2002). *Anopheles gambiæ* tends to take blood-meals especially at dusk (Clements, 1999) and, being anthropophilic (Constantini *et al.*, 1998), *An. gambiæ* is commonly found at these times inside houses. After feeding on blood, *An. gambiæ* can be found resting on the walls of houses during the night and in the morning. Even in the morning, ambient light levels tend to be low in many African houses where electricity is not widely available. Perhaps, in these low-light situations, the ability to use olfactory cues is especially important, even if backed up by acute vision at close range.

(Photo of lake flies on *Lantana camara* courtesy of Robert Jackson)

- *Evarcha culicivora* can distinguish between different kinds of plants on the basis of odour. This is the first salticid for which a preference for a particular plant has been demonstrated experimentally. The biology of *E. culicivora* appears to be linked to *Lantana camara* in particular (Chapter 8).

Although many jumping spiders are known to feed on nectar from plants (Ruhren & Handel, 1999; Jackson *et al.*, 2001b), no salticid had been directly linked to any particular species before the research on *Evarcha culicivora*. In the field, *E. culicivora* appears to be especially often found on *Lantana camara* (a common shrub in its habitat). It feeds on the nectar produced by *L. camara*, as well as on the insects that are, themselves, feeding on the nectar of *L. camara* (RRJ, pers. comm.). In laboratory experiments, *E. culicivora* distinguished the odour of these plants from the odour of other plants (Chapter 8), and showed a preference for the odour of *L. camara*. *E. culicivora's* preference for the odour of this plant may indicate that it uses odour to locate the plant from a distance, before visual cues from the colourful flowers become noticeable to the spider.

L. camara may be a haven (Gujral & Vasudeval, 1983; reported in Day *et al.*, 2003) and nectar source (McCrae *et al.*, 1968; 1969; 1976; reported in Clements, 1999) for mosquitoes. Male mosquitoes do not feed on blood. Instead, they survive by feeding on nectar. It is becoming increasingly evident that nectar is also important for female mosquitoes. Even mosquitoes that need blood to complete their gonotropic cycle tend also to feed on nectar (Clements, 1999). Females of

An. gambiæ feed on nectar for their initial host-seeking flight (Takken & Knols, 1999; Foster & Takken, 2004). Pertinently, Gary & Foster (2004) and Impoinvil *et al.* (2004) independently established that the nectar from *Ricinus communis*, and, to a lesser extent, *Lantana camara*, has a beneficial effect on *An. gambiæ's* longevity.

Feeding on nectar between blood meals may extend the female mosquito's lifespan (Gary & Foster, 2004; Impoinvil *et al.*, 2004) and, therefore, the potential egg-producing capacity of *An. gambiæ*. This may have implications for the control of malaria. Females of *An. gambiæ* that have fed on blood and sugar (10% sucrose solution) survive longer than females that have fed on either blood or on 10% sucrose solution alone (Gary & Foster, 2001). Females of *An. gambiæ* that have fed solely on blood bite more often and have a higher daily fecundity than either mosquitoes that have fed only on sugar or mosquitoes that have fed on a combination of blood plus sugar. However, the increased survivorship of females that have been on a diet of blood and sugar offsets the differences in daily fecundity. In warm climates, this may not be especially important, because even females fed on blood alone tend to live long enough (median 18 days) to be effective malaria vectors. Whether it is advantageous to have plants that provide mosquitoes with nectar near human habitation is uncertain, because the decrease in *An. gambiæ's* biting frequency may be offset by the increased longevity of the mosquito. However, if, as anecdotal evidence suggests (McCrae *et al.*, 1968; 1969; 1976; reported in Clements, 1999; Gujral & Vasudeval, 1983; reported in Day *et al.*, 2003), *An. gambiæ* is attracted to *L. camara*, *E. culicivora's* role in the biological control of malaria may be more complex and more effective than simply having them in houses. The jury is still out.

Insect attraction to plants has been cleverly devised as a possible control method for the sand fly-borne disease leishmaniasis. Sand flies, like mosquitoes, supplement their blood diet with plant-derived sugars (Jacobsen *et al.*, 2001). By baiting sugar sources with the larvicide *Bacillus sphaericus*, adult sand flies (*Phlebotomus papatasi*) may act as carriers of the larvicide to the larval habitat, causing a significant increase in larval mortality (Robert *et al.*, 1997). In addition, Schelin *et al.* (2001) showed that sand flies feed by preference on nectar from certain plants (including *R. communis*) which, paradoxically, shortens the life-span of the sand fly. The authors argue that because the plants to which *P. papatasi* is attracted are not native to Israel, where this research was done, the sand fly has not yet evolved mechanisms to cope with the negative effect of the plants (Schlein *et al.*, 2001). If *L. camara* is indeed a haven for *An. gambiæ* mosquitoes, precise knowledge of what attracts the malaria vector and its predator, *E. culicivora*, to these plants could be of some considerable importance.

Being attracted to the nectar of *L. camara* may be, for *E. culicivora*, an adaptation by which it gains a nutritional supplement (probably primarily sugar) (see Pollard *et al.*, 1995). We do not know whether the nectar from this particular plant is nutritionally more advantageous to *E. culicivora* than the nectar of other plants. *L. camara* is known to send chemical signals to its pollinators (Gardener & Gillman, 2002), and possibly *E. culicivora* is one of the pollinators of this plant. Another possibility is that *E. culicivora* is attracted to a chemical signal produced by plants under herbivore attack to attract predaceous arthropods (Weissbecker *et al.*, 2000; Hammack, 2001). However, *E. culicivora's* attraction to *L. camara* as a predaceous arthropod would appear to help the plant minimally because *E. culicivora* appears to feed primarily on mosquitoes and mosquitoes are unlikely to harm *L. camara*. If *E. culicivora's* preferred prey, blood-fed mosquitoes, is especially often found on *L. camara*, then the primary advantages *E. culicivora* gains from seeking out *L. camara* might be to find its prey. However, none of these hypotheses can be critically assessed at this stage. A great deal of additional research is urgently needed on this apparently unique spider-plant system.

- Investigations were made into how *Evarcha culicivora* behaves in the presence of *Lantana camara*. *E. culicivora's* prey-choice decisions in the presence of chemical cues from *L. camara* are differed from its prey-choice decisions in the absence of chemical cues from *L. camara*, and further work showed that specifically the presence of β-caryophyllene, a volatile produced by *L. camara*, affected *E. culicivora's* prey-choice decisions (Chapter 9). In the presence of *Lantana* flowers and of β-caryophyllene, *E. culicivora* did not appear to discriminate between different mosquito prey that were discriminated between in the absence of odour. Furthermore, in the presence of *Lantana* flowers and of β-caryophyllene, *E. culicivora* did not appear to discriminate between virtual mosquitoes and virtual lake flies (made using 3D computer animation) that were discriminated between in the absence of odour (Chapter 10). This is the first demonstration of a behavioural change induced in a spider by chemical cues from plants.

The results presented in Chapters 9 and 10 were surprising. This most selective of predators, a salticid that usually chooses to feed on blood-fed female mosquitoes above all else, became indiscriminate about its choice of prey when in the vicinity of the odour of *L. camara* (Fig. 4). A specific sesquiterpene, β-caryophyllene, was shown to have this effect, although the possibility that

others of the dozens of compounds present in *L. camara's* headspace (see da Silva *et al.*, 1999; Ngassoum *et al.*, 1999; Kahn *et al.*, 2002; Sefidkon, 2002) may also influence *E. culicivora's* behaviour cannot be ruled out.

A number of studies have demonstrated the importance of sesquiterpenes in the signalling systems of insects, with antennae of many insects responding to β-caryophyllene (Zhu *et al.*, 1999; Weissbecker *et al.*, 1999; 2000; Al Abassi *et al.*, 2000; Kalinová *et al.*, 2000; Bichão *et al.*, 2003). However, the earlier example of the effects of β-caryophyllene were all of herbivorous insects being attracted to host plants for food and oviposting or of predaceous insects being attracted to plants to find prey (i.e., to find the herbivorous insects that are attacking the plant). Nothing has been reported like the effects demonstrated here for *E. culicivora*.

Although β-caryophyllene seemed almost like a mind-altering drug in its effect on the spider, making it stop exercising its prey preferences, β-caryophyllene did not appear to simply inhibit the spider's motivation to attack prey. Perhaps being a 'drug-crazed spider' when on *Lantana* is a side-effect of *E. culicivora* seeking out *L. camara* for some other purpose. For example, the odour of *L. camara* may put *E. culicivora* in a state of readiness to feed on nectar, and *E. culicivora* may have temporarily shut down the processing system used for discriminating between different kinds of prey. If *L. camara* is a rich source of prey, relative to the prey to be found everywhere else, then, given that *E. culicivora* still attacks prey (even if in a non-discriminating manner), the net effect on *E. culicivora* may be a positive one.

Figure 4. Hypothetical question that *E. culicivora* can be considered to address before stalking its prey in the vicinity of flowers of *Lantana camara*. Photo of male *E. culicivora* on *Lantana camara* courtesy of Robert Jackson.

I sometimes pause and marvel that *Evarcha culicivora* responded to my virtual mosquitoes. Then I get carried away, thinking of all the things that could still be done. The success of animation techniques with *E. culicivora* opens up fascinating new avenues for research on behaviour, perception and decision-making.

More work on colour discrimination by *E. culicivora* stands out as especially important. The next step might be choice tests (as described in Chapter 7) in which the control is a mosquito with a red abdomen and the other mosquito's abdomen is one of an array of other colours (orange, yellow, green, etc.). Any of these can then be paired-up to make fine distinctions (light green/dark green, dark green/dark red). However, in my view the most important of these tests is to compare two long wavelengths, red and green.

Colour combination tests using *E. culicivora* of different size categories might be carried out systematically. In this way, we might learn not only about the ability of *E. culicivora* to discriminate between colours, but how this ability may change with an increase in the size of the anterior-median eyes.

Other tests using colour might explore whether red is a supernormal stimulus for *E. culicivora*. As a start, choice tests might be carried out in which one mosquito that has a red abdomen is coupled with another mosquito that has both a red abdomen and a red thorax (or something else that is not red in a real blood-fed mosquito: legs, head). *E. culicivora* might prefer a supernormal mosquito to the more realistic virtual prey.

Animation methods empower the experimenter to new avenues of research. As I have shown, with this method optical cues can be presented simultaneously, and in highly controlled conditions, with chemical (or other) cues. Needless to say, these methods could be very useful for studying chemoreception, with priming effects being readily explored. Studies integrating the reception of sensory information with studies of the spider's decisions may provide an especially powerful integrative approach to animal behaviour.

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APPENDIX I
Animation methods

Introduction

This appendix provides details of the methods which were used for producing the virtual prey in Chapters 4, 5, 7, 9 and 10. Instead of tedious and confusing textual descriptions, this is a pictographic overview of the production of animations. This overview illustrates the basic methods used and the level of detail that was achieved. The appendix includes information about the pictures of the insects that drawings were based on as well as the process of drawing and animation.

PC-based software (3D Studio Max or 3Ds) was used. First, detailed images of the animals, *Anopheles gambiæ* and *Chaoborus* sp., were needed and for this I relied on microscopy images taken from several perspectives (Figs. 1-3). Morphological details about *Anopheles gambiæ* were taken from Huang (2001) (see also Chapter 2). Insects used for microscopy were asphyxiated with CO_2 and then placed in 80% ethanol in the field site, Mbita Point (Kenya). All microscopy work was done in New Zealand using a Leica MZ12.5 stereomicroscope with a Planapo 1.0x objective. Images were captured at a resolution of 1300(h) x1030(v) pixels from the microscope using a Zeiss AxioCam HRc CCD digital camera and transferred to Zeiss AxioVision 3.1 software. Because of the many ways of zooming into the image with this setup, rather than providing numerical magnifications, a 1mm scale bar is shown on each image. Animals being photographed were lit from above using a Schott KL1500 cold light source and from underneath using a Leica SLS 150x cold light source.

Once the 3-D virtual *Anopheles gambiæ* had been drawn, it was animated in grooming behaviour. Frame-by-frame analysis of digital video footage of mosquito grooming behaviour was used as the template for the animation sequence.

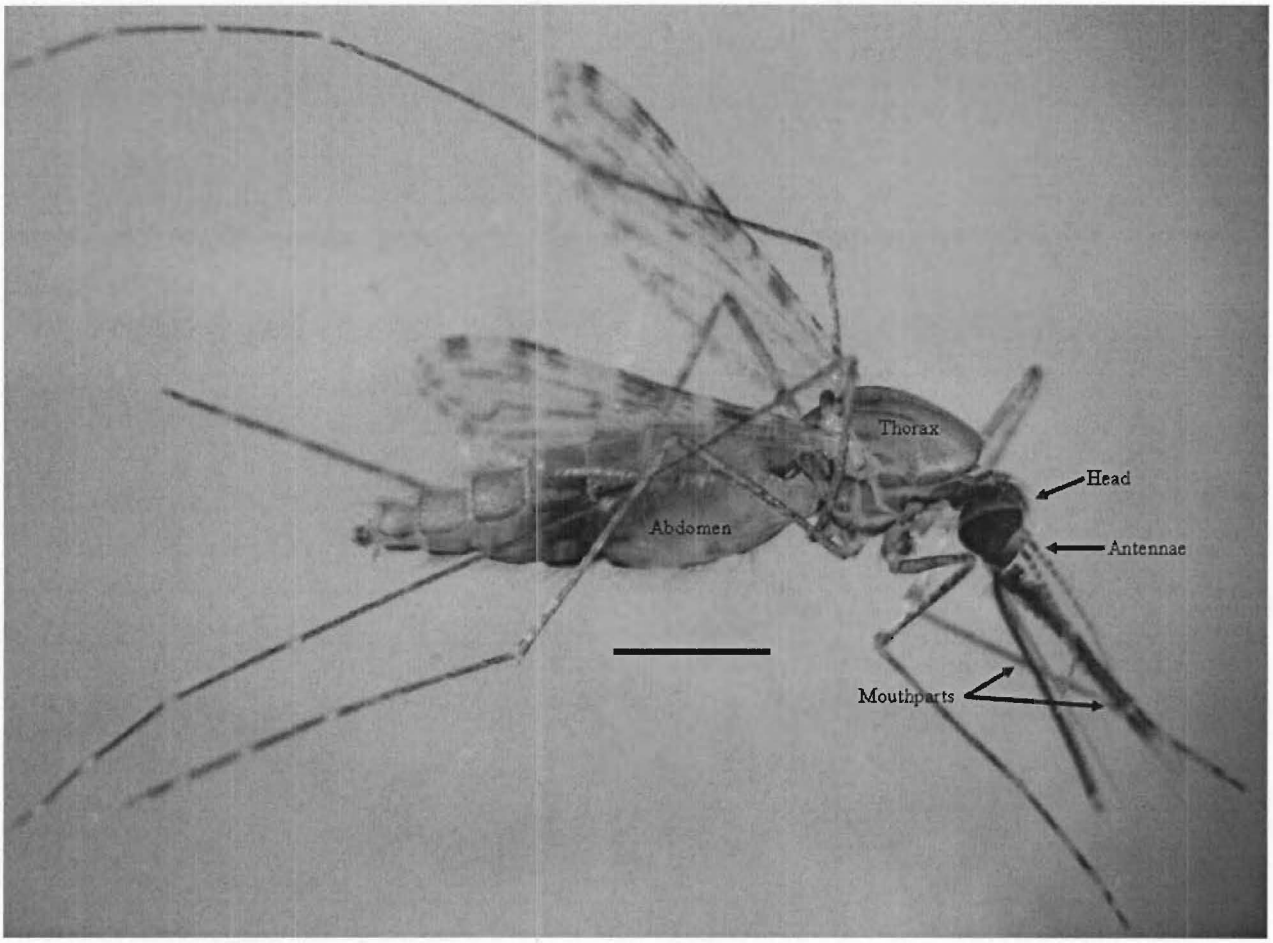

Figure 1. Microscopy image of blood-fed female *Anopheles gambiæ*. Lateral view. Note antennae without obvious whorls of setae ('bare').

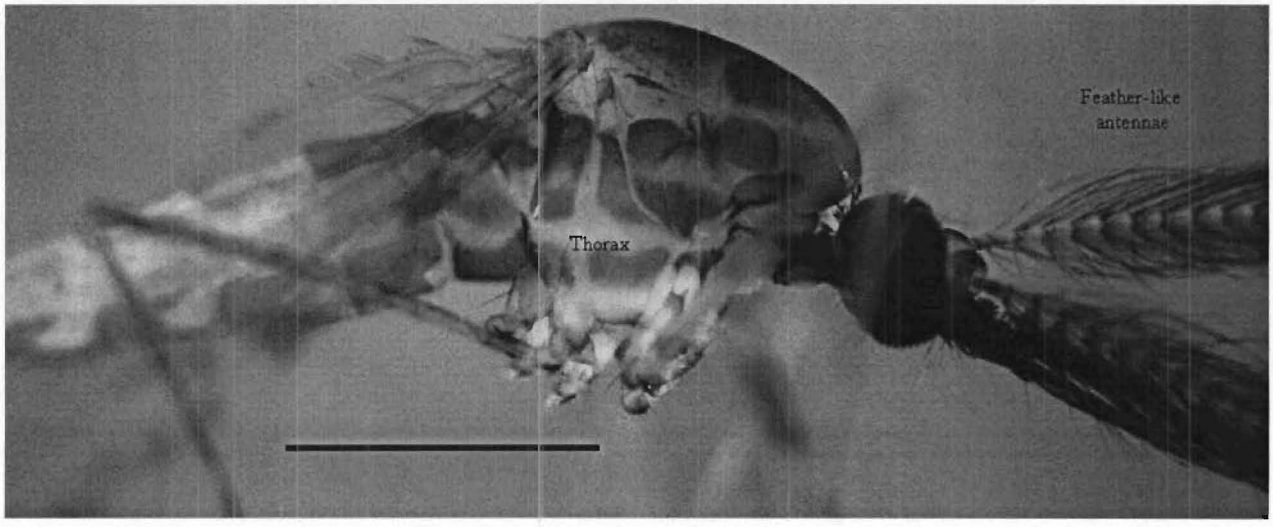

Figure 2. Microscopy image of thoracic region of male *Anopheles gambiæ*. Lateral view. Note the feather-like antennae.

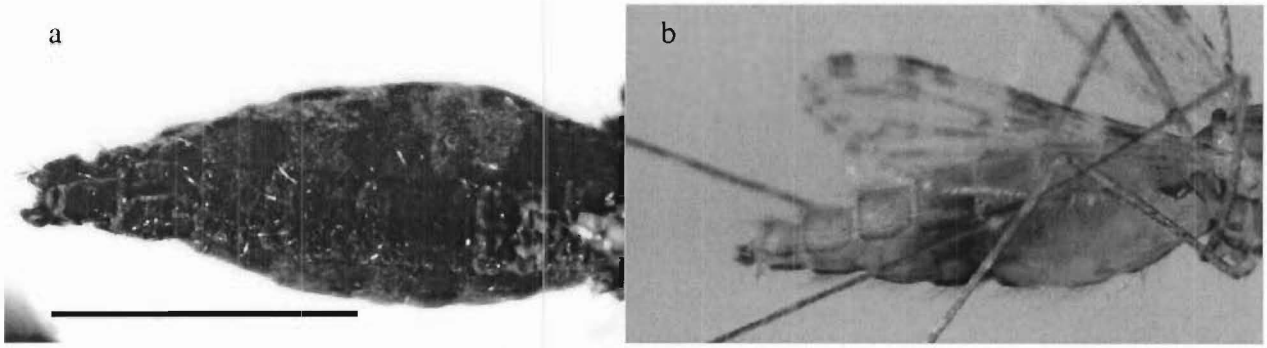

Figure 3. Microscopy images of the abdomen of blood-fed female *Anopheles gambiæ*. **a)** Ventral view. **b)** Lateral view. Note engorged shape of abdomen.

To draw the abdomen of the blood-fed female *Anopheles gambiæ*, I used one of several available drawing techniques in 3Ds. Initially, a 3-D line-mesh, called a spline, was drawn by hand (Fig. 4) using the image shown in Fig. 3b as a template for the lateral view. The combination of a lateral view, a ventral and a dorsal view are necessary for accurate rendition of the 3-D shape of the mosquito's abdomen. Polygons of the spline can be highlighted and shaped independently (Fig. 4). However, lines, edges, vertices and surfaces can be also selected for shaping (right-hand side of the screen shown in Fig. 4; polygons currently selected and highlighted in yellow). Another method for drawing (deforming and altering shapes available in 3Ds' pull-down menu shown in Fig. 4), was used for creating the head and eyes of the mosquito (Fig. 5) and this was based on a microscopy image of the head of an *Anopheles gambiæ* (Fig. 6).

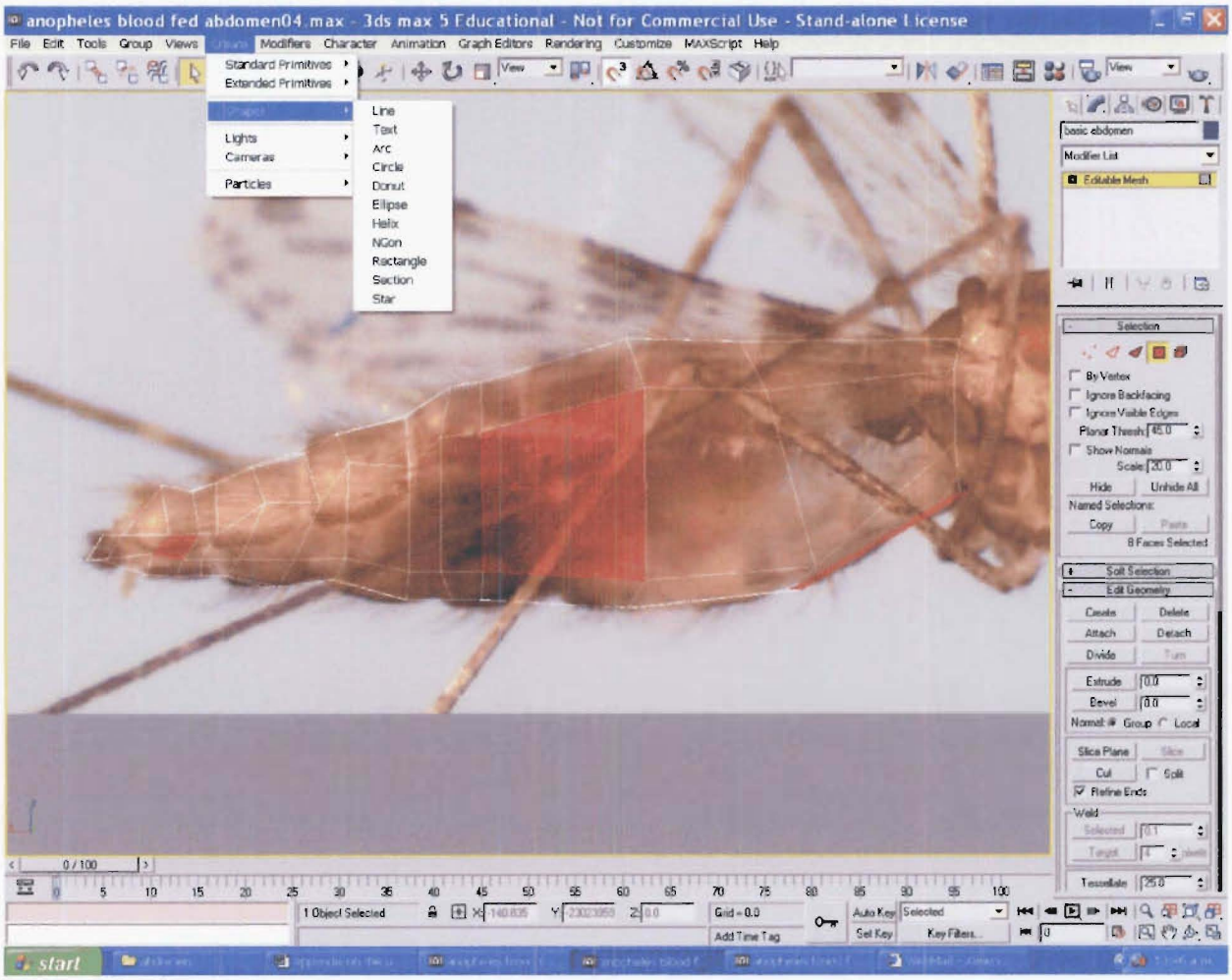

Figure 4. Lateral view of hand-drawn 3-D spline of abdomen of a blood-fed female *Anopheles gambiæ* in 3Ds with three highlighted polygons. Note pre-existing shapes available in pull-down menu of software.

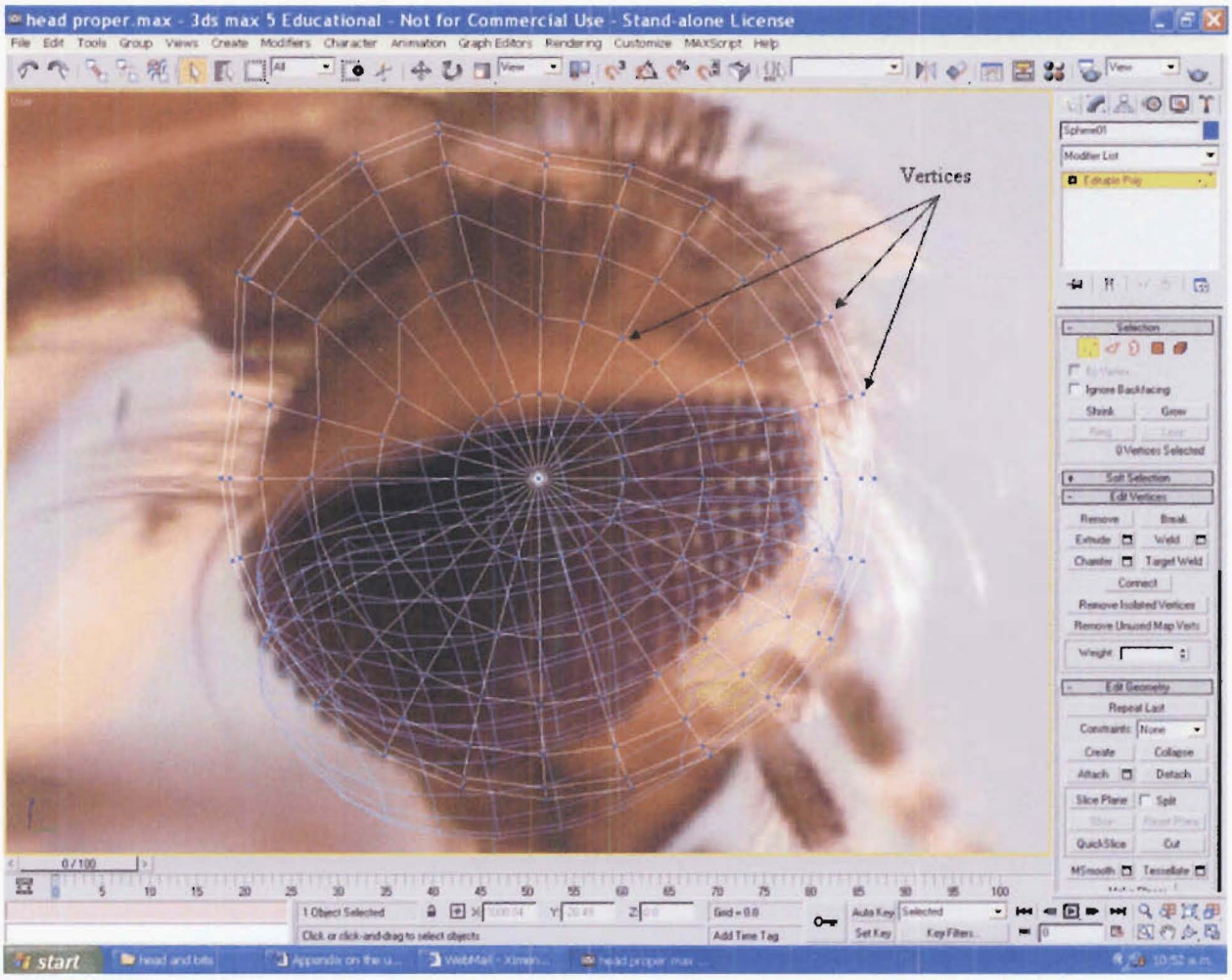

Figure 5. User or perspective view of the shapes that will become the eyes and head of the virtual mosquito.

The shapes used to produce the head and eyes of the mosquito (Fig. 5) were simple spheres that were deformed and shaped principally at the level of vertices (highlighted in yellow on right-hand side of the screen shown in Fig. 5). When the vertices of a particular shape are selected (blue dots, Fig. 5) they can be pulled and moved individually to produce the correct shape. In a 3-D image that is symmetrical the vertices will lie directly behind one another, so care must be taken to select only one vertex at a time because seemingly selecting one vertex may actually select all vertices along that particular plane. Because of these difficulties a perspective view can be helpful, as illustrated in Fig. 5, where both eyes can be distinguished independently with a careful look at the vertices of the globular shapes that form the eyes.

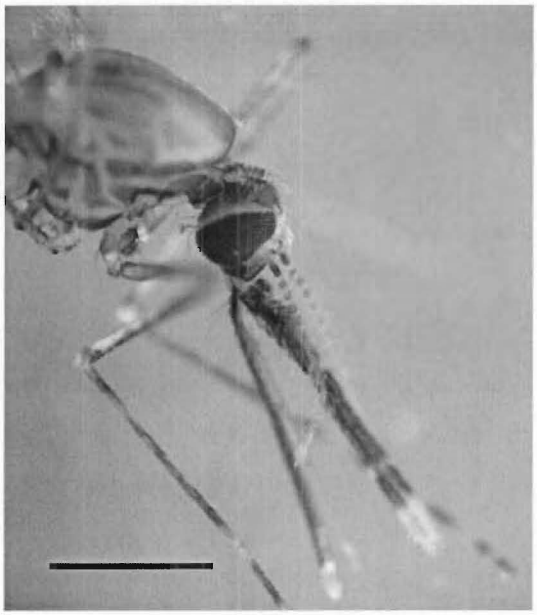

Figure 6. Microscopy image of head and thorax of female *Anopheles gambiæ*. Lateral view.

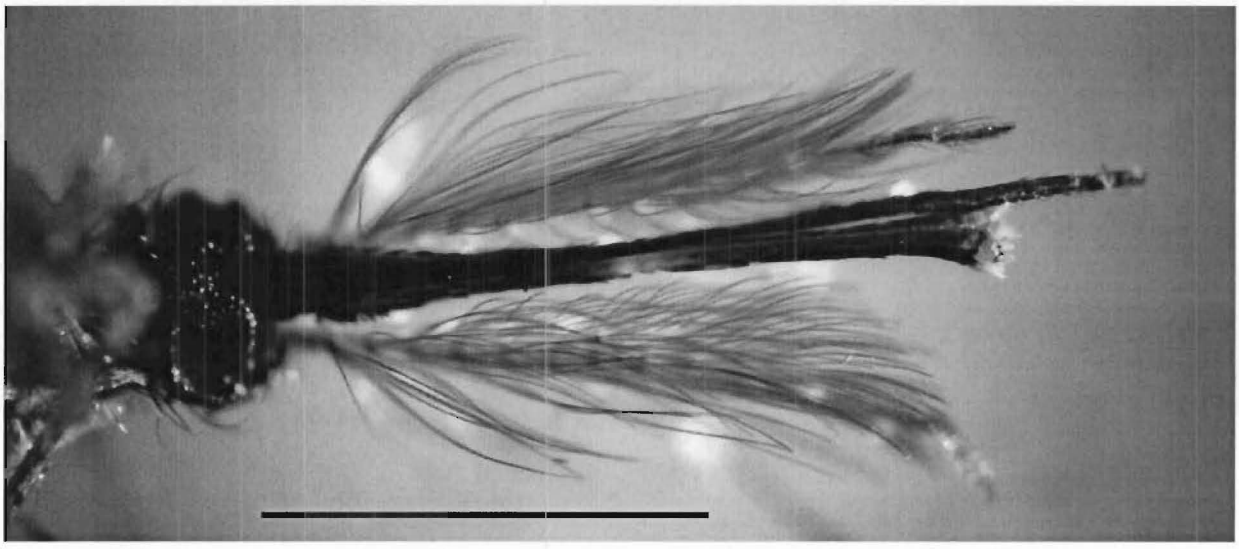

Figure 7. Microscopy image of male *Anopheles gambiæ* head region. Ventral view.

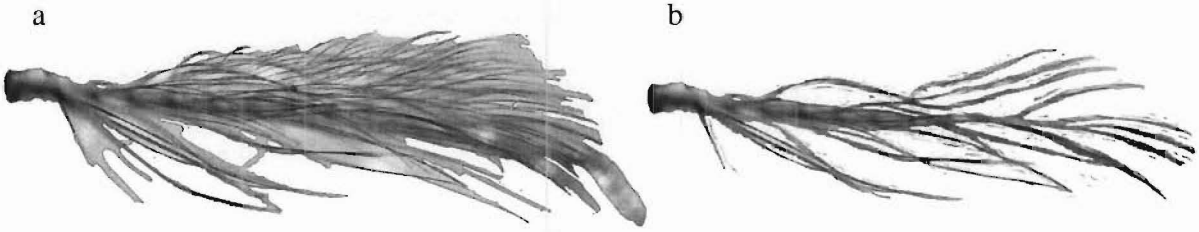

Figure 8. Antennae used on virtual mosquitoes. **a)** Male antenna. **b)** Female antenna.

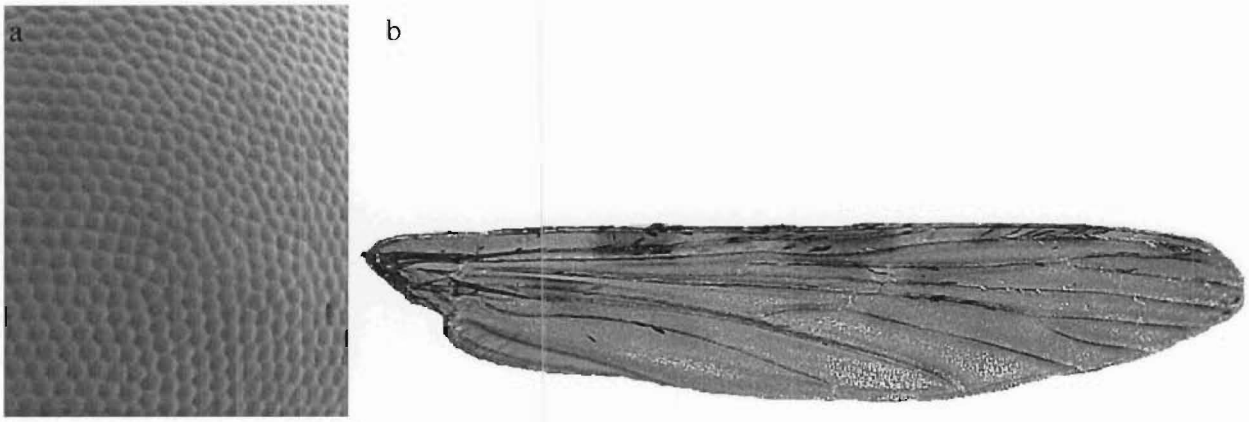

Figure 9. Materials used to wrap on areas of virtual mosquitoes for added realism. **a)** SEM of mosquito compound eyes used as material for eyes of virtual mosquitoes. From: Museum of Science, Boston (http://www.mos.org/sln/sem/sem.html; accessed 23/10/02). **b)** Edited image of *Anopheles gambiæ* wing used as actual wing on virtual mosquitoes.

Drawing antennae for virtual mosquitoes was not a feasible alternative because the resolution of one pixel (the narrowest line width) was inadequately wide. Instead, a photo of an antenna (bottom antenna of Fig. 7) that had been edited to remove the background (Fig. 8) was placed on a transparent 'box' which was then twisted and bent to give it a 3-D appearance. The box was placed on the head of the virtual mosquito. The antenna was placed on the deformed box as a 'material map', taking into account the coordinates of the shape that the material was wrapped around. The coordinates provide the software with the information necessary for it to calculate how to orient and scale the material onto a particular shape. For example, a scanning electron microscope image of the compound eyes of a mosquito downloaded from the internet (Fig. 9a) was used to give the virtual mosquito's eyes more realism. The material was 'shrink-wrapped' over each eye assuming spherical coordinates. Materials can be manipulated in a variety of ways, such as increasing reflection and altering the colours. One particularly useful manipulation is that of 'bump', in which materials can be given a bumpy texture. The amount of bump given to a material can be specified numerically. For the material used in the eyes of the virtual mosquitoes, the surface of material was then given a specified amount of bump to increase the 3-D realism of the compound eyes.

Drawing realistic wings posed difficulties similar to those experienced when drawing antennae. Wing venation differs between mosquito species (Huang, 2001) and the veins themselves

are extremely thin, so the best option for accuracy and realism was to use an image of a real wing (Fig. 9b). Because there was no need for the wings to be three-dimensional, the image of the wing was not placed on a transparent box (as the antennae were), but was merely shaped to the contour of the body of the virtual mosquito (by giving the image some curve) and placed on top of the abdomen (Fig. 10).

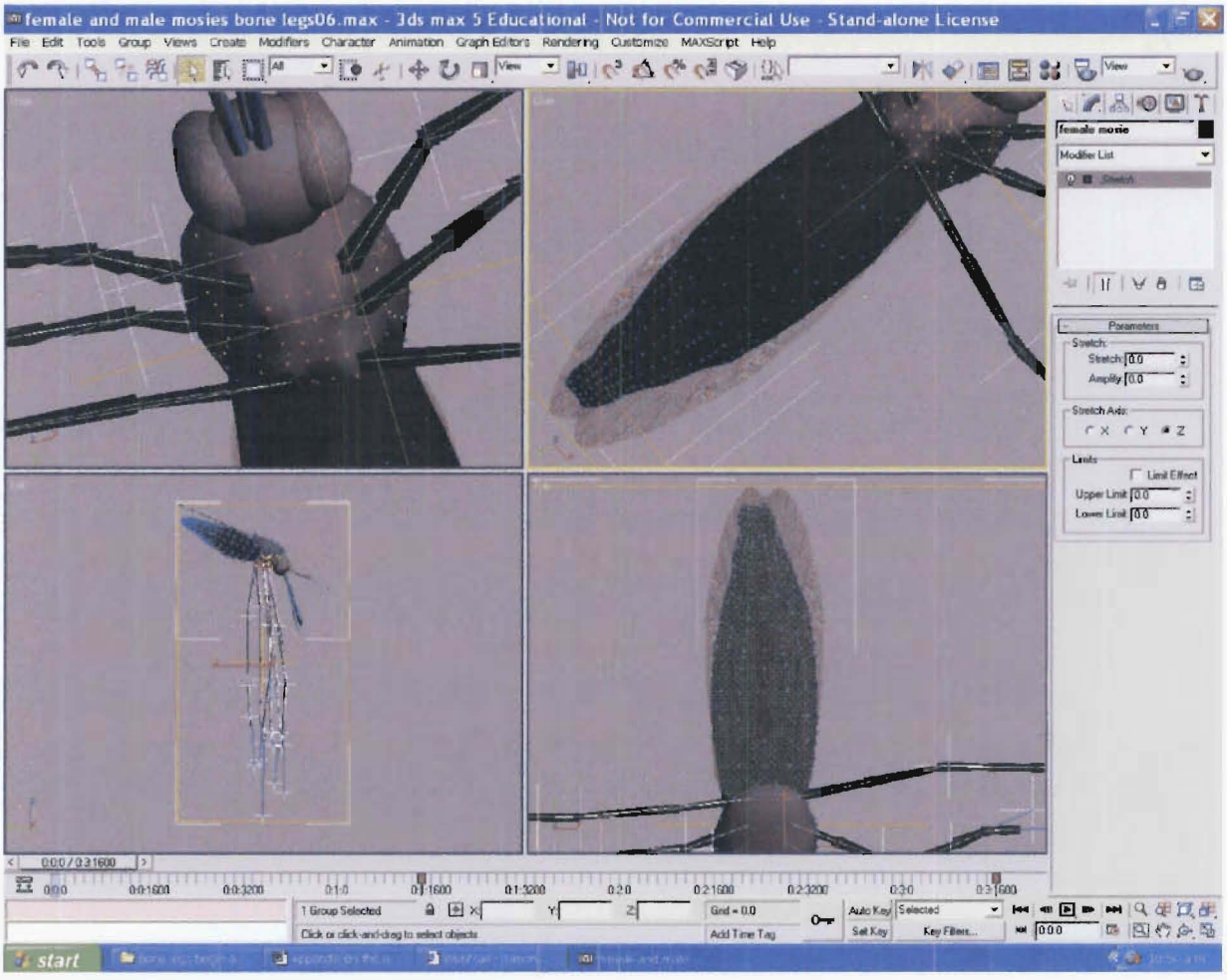

Figure 10. Typical screen-view in 3Ds. Four windows, each showing a different aspect of the three-dimensional drawing.

Fig. 10 shows the mosquito before animation. On the top-left user window is the thorax and head of the virtual mosquito seen from a ventral perspective. In this screen the vertices of the thorax that have been 'soft selected' are clearly visible. By soft selecting vertices these can be deformed as a group from higher (red) to lower (yellow) intensity, rather than individually. Vertices can be skewed, stretched and otherwise deformed in several ways. In this case 'stretch' has been selected as

the method for deforming the vertices (right-hand side of screen, Fig. 10). The material for the mosquito's eyes (Fig. 9a) has been placed on the eyes but does not look realistic because the mosquito has not yet been rendered. Rendering (i.e., producing the final image with the appropriate calculations for light and texture) is the last stage of the process, taking place only after animation. Before animating, the virtual mosquito must be placed in its 'starting' posture. Fig. 10 shows the entire mosquito before the legs have been placed into a natural resting posture from which the animation will be based (bottom left window). The white crosses on the legs serve as pulleys with which to move the legs at each joint. Once the legs have been moved and the body placed in a satisfactory starting position (in this case a typical anopheline resting position), the virtual mosquito is ready to be animated (Fig. 11).

Moving six legs, each with several joints, can be complicated. For this reason, it is sometimes useful to view the mosquito 'mesh' in different ways, as illustrated in Fig. 11. The mosquitoes in the top-left and bottom-right windows are viewed with 'edges' so both the mesh and the filled-in drawing are visible, whereas the top-right window shows the 'wire-frame' view in which only the mesh is visible. In the bottom-left window, the mosquito is seen in the 'smooth' view, in which the mesh of the shapes forming the objects are not shown and only the filled-in objects can be seen. Smooth views are useful for a quick view of what the image will look like but cannot be used when deforming objects.

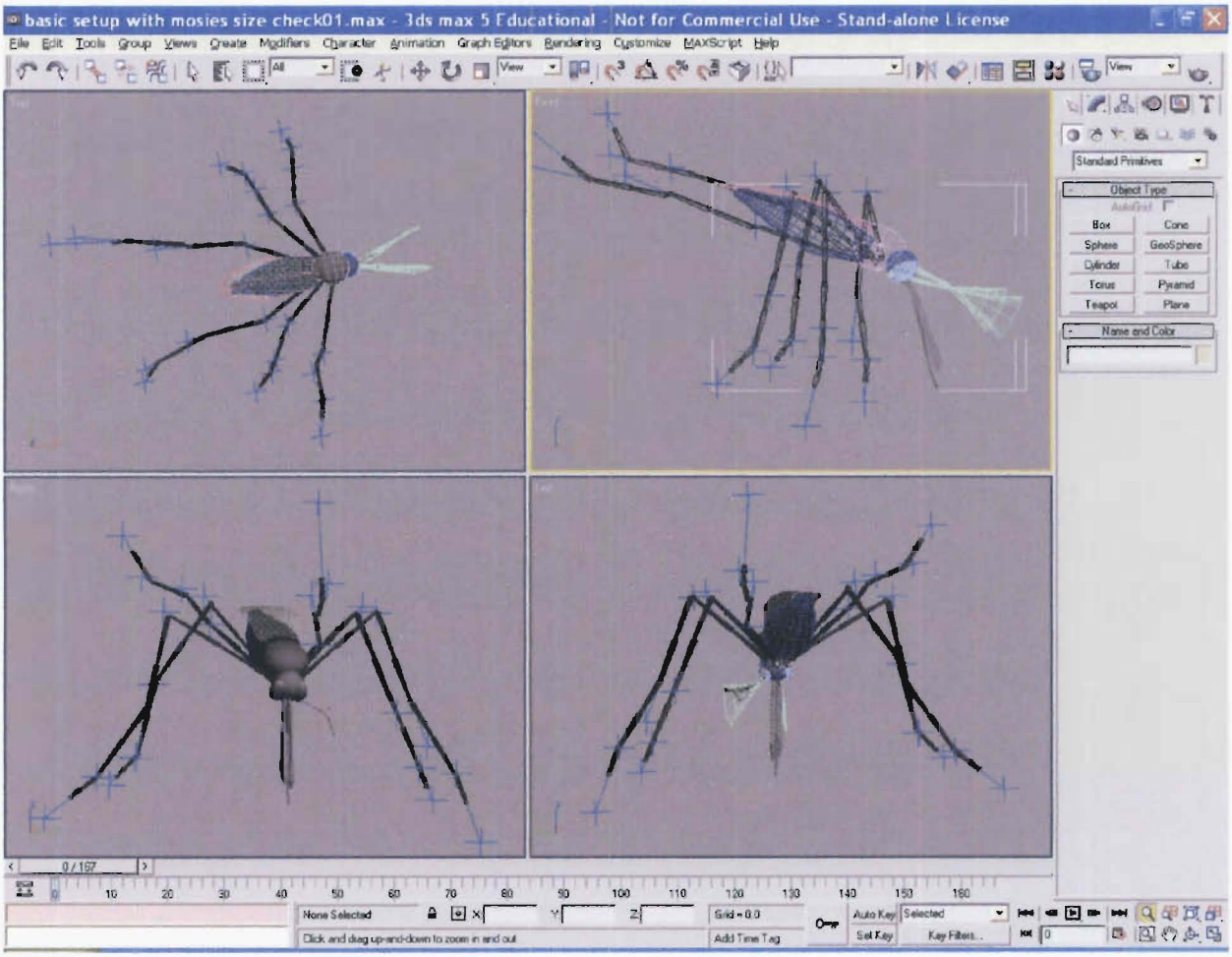

Figure 11. Dorsal, lateral, frontal and posterior views of virtual anopheline in basic 'resting posture'.

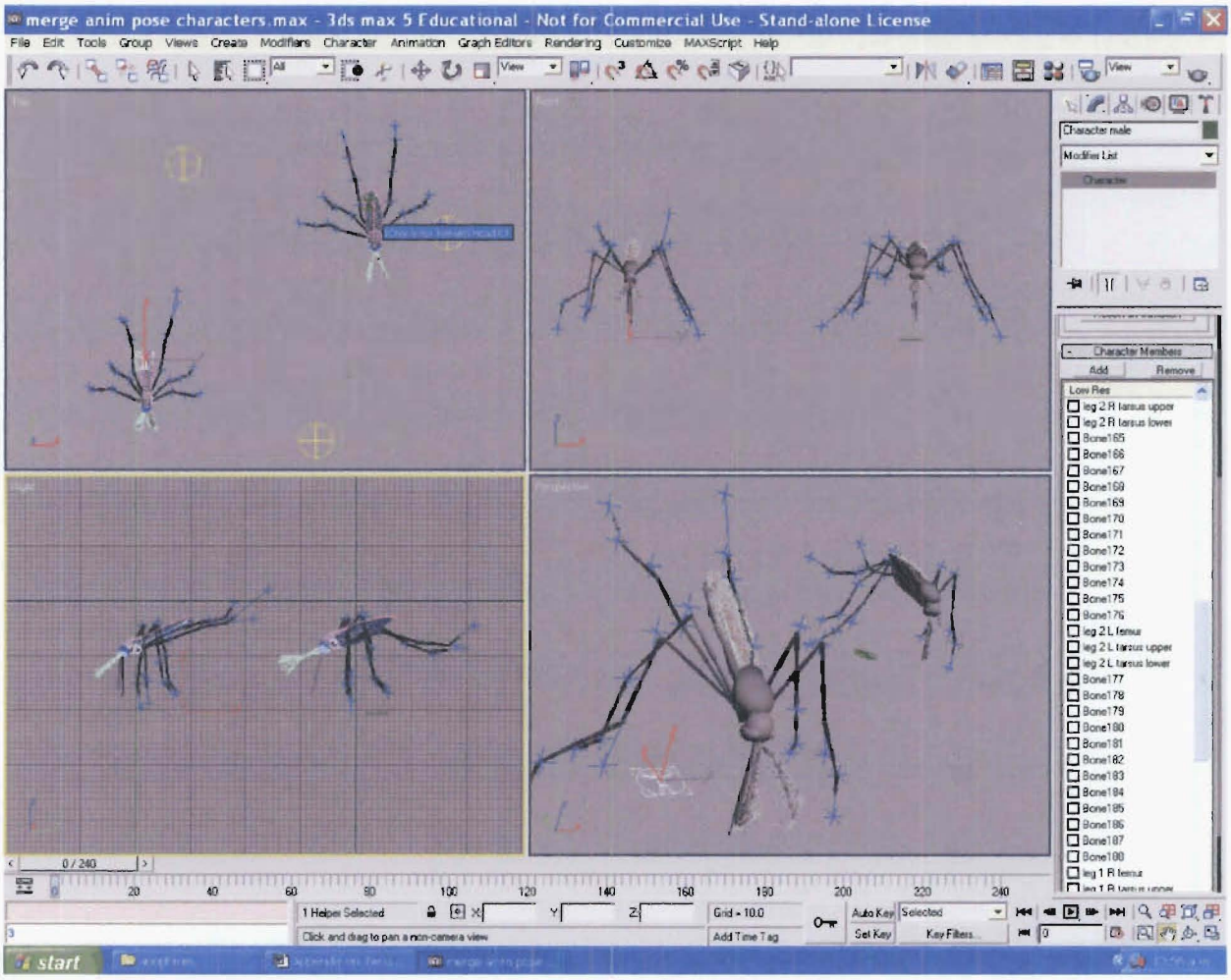

Figure 12. Male and female mosquitoes being animated. Before animation each mosquito is assigned a character (circles under mosquitoes) that tells the software all the items that compose the one unit for animation (far right). Scene contains lights (yellow circles).

Once the mosquitoes are in their resting posture, ready to be rendered, the scene needs to be set. It is important to get enough light and, for virtual mosquitoes, it is important that the light appears to be natural, ambient light (not indoor-type lighting). The three yellow circles in top-left window of Fig. 12 are ambient lights that were placed in the scene (once rendered these circles are not visible).

The next step before rendering is to create a so-called 'character'. The character identifies the entire mosquito to the software. Each mosquito consists of many different shapes and hundreds of 'bones' in the legs (Fig. 12, right-hand side of the screen). For the animation to work smoothly, the computer has to be told that all these items form part of a whole (the character of the mosquito).

When the character is selected (white highlighted square with a circle inside it, best seen in the bottom-right window in Fig. 12) and parts of the character (e.g., legs of the mosquito) are moved during animation the rest of the character moves in synchrony. The animation toolbar seen along the bottom of Fig. 12 shows the frames of the animation sequence. In Fig. 12 the scene is on frame 0 of 240. To animate the mosquito, the legs were moved based on a frame-by-frame analysis of a digital video sequence of mosquito grooming behaviour.

Once the animation for a character has been completed it can be transferred to another character. In this way, the many mosquitoes drawn for experiments (Fig. 13) are all animated to move in exactly the same way and at exactly the same time, ruling out any movement-related confounding factors in tests (Fig. 13f).

After animating, the computer has to render each frame of the animation. In other words, the lighting, materials and movement in every individual frame have to be calculated to produce the final movie output in the form of an AVI file which can then be played by most PC-based multimedia software packages, such as Windows Media Player or QuickTime. Rendering is slow, taking 2-3 h to render 240 frames. The animation was created at 25 frames/s, meaning that it takes almost 3 hours to create 10 s of movie file on a PC. Because of this, the movies that were played in experiments were short (240 frames), but they were set to repeat, or loop, after finishing. In this way, movies that I used for 15 min tests did not take days to render and did not crash the computer while loading!

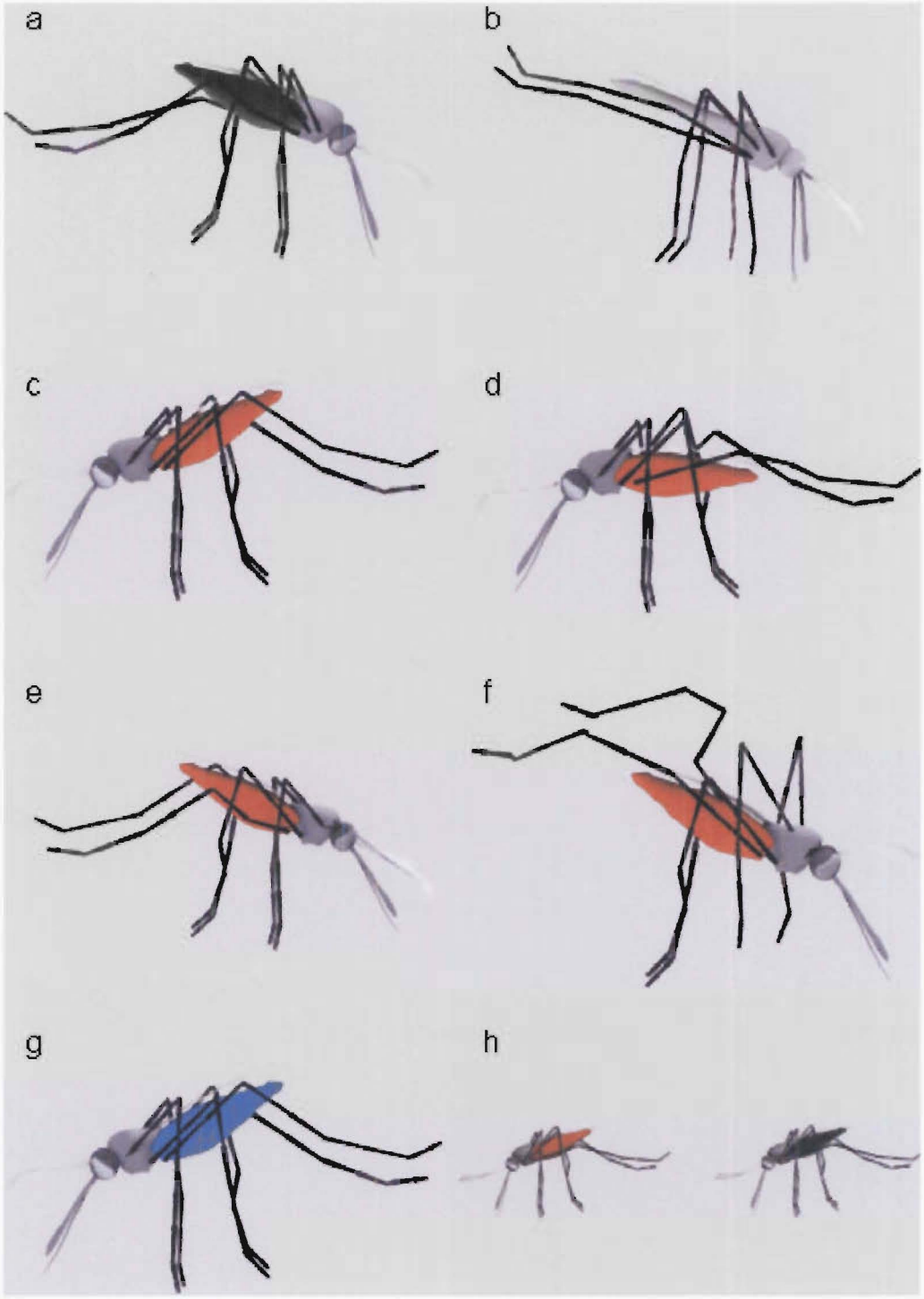

Figure 13. Detailed view of the anopheline mosquitoes that were drawn for choice tests with virtual prey. **a)** Greyscale blood-fed female. **b)** Greyscale male. **c)** 'Red' blood-fed female, identical to (a) but with colour added to the abdomen. **d)** 'Horizontal' posture blood-fed female. **e)** Blood-fed female with male antennae. **f)** Blood-fed female with 'odd' movement. **g)** 'Blue' blood-fed female. **h)** Basic choice test with two blood-fed females: one red and one greyscale.

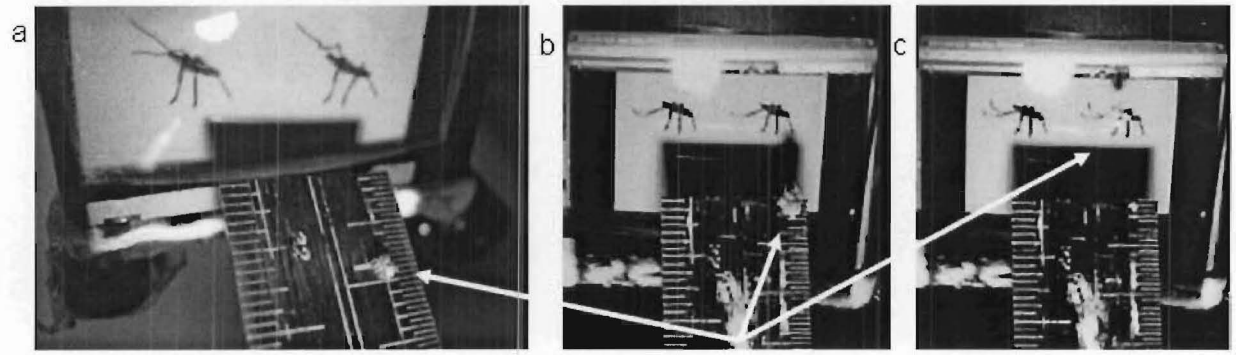

Figure 14. Images of experimental set-ups with *Evarcha culicivora* juveniles. **a)** Normally moving blood-fed female (left) and oddly moving blood-fed female mosquito. Spiderling standing at *c.* 1 cm from end of ruler (right) watching screen. **b)** Frame from video sequence of jumping juvenile *Evarcha culicivora.* Spider poised to leap at end of ramp (right) in front of red, not greyscale (left) blood-fed female. **c)** Same spider, having jumped on its prey, obscuring abdomen of 'red' blood-fed virtual mosquito.

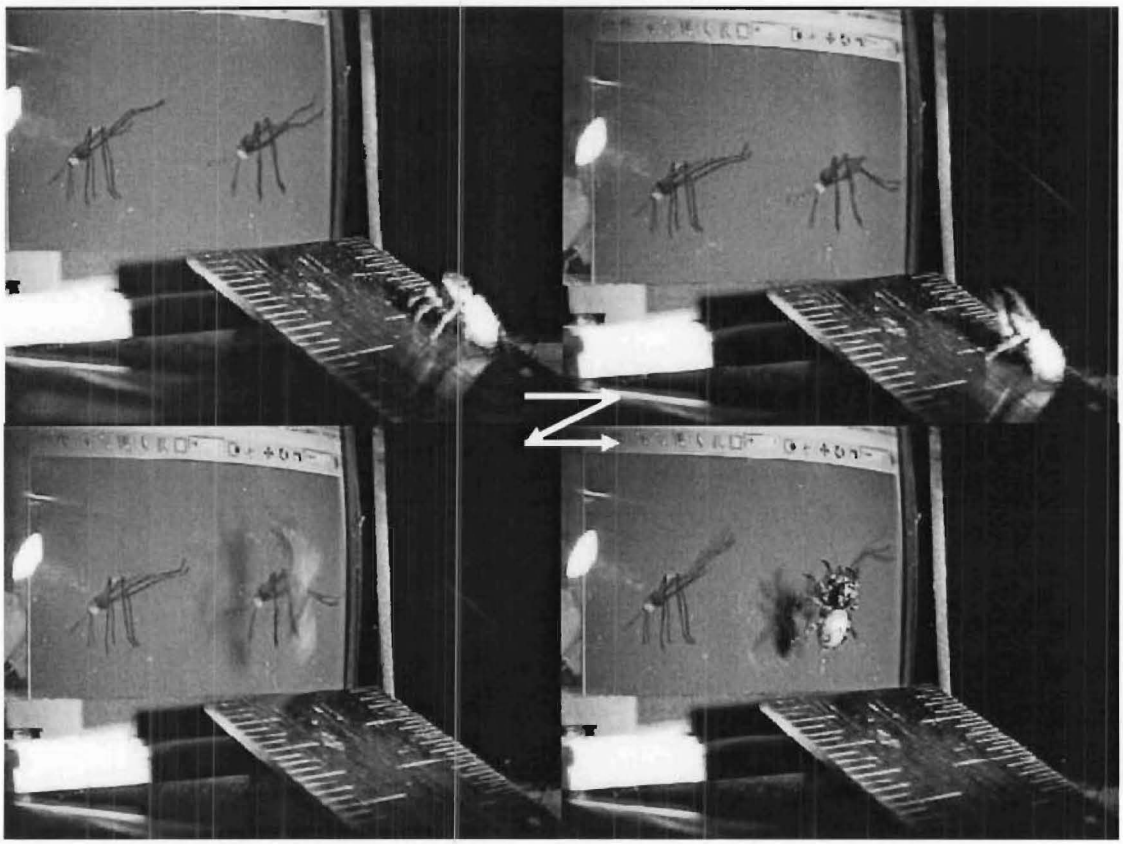

Figure 15. Selected frames from digital video sequence of adult female *Evarcha culicivora* leaping on greyscale blood-fed female mosquito. Other prey: greyscale male mosquito. Spider lands directly on prey.

Lake flies

The process for drawing non-biting midges (chaoborids), or 'lake flies', was the same as that used for drawing mosquitoes. Firstly, the abdomen was drawn. Unlike with the abdomen of the female mosquito, I deformed basic shapes (cylinders) to draw each segment in the abdomen of the lake fly (Fig. 16). After tapering and extruding each segment so that they matched the photographic template (Fig. 17a), individual segments were 'welded' together to form a single unit. The abdomen was based on a combination of a microscopy image of a lake fly (Fig. 17a) and on a photo (courtesy of Robert Jackson) taken with a Nikon F4 SLR camera with a macro lens which was then scanned at 4000dpi on a Nikon Coolscan 4000 (Fig. 18a).

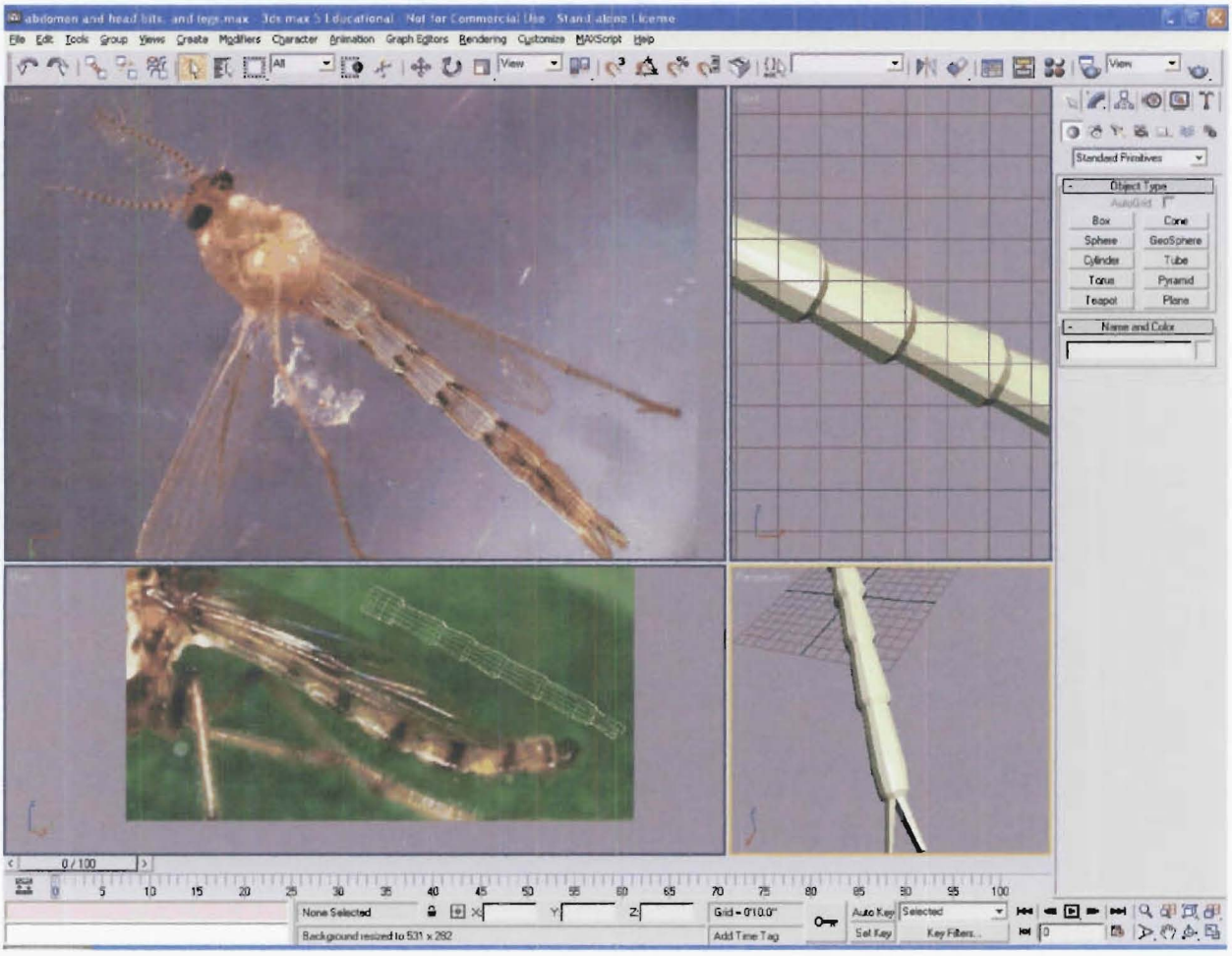

Figure 16. Different views of segmented abdomen of lake fly at various levels of detail.

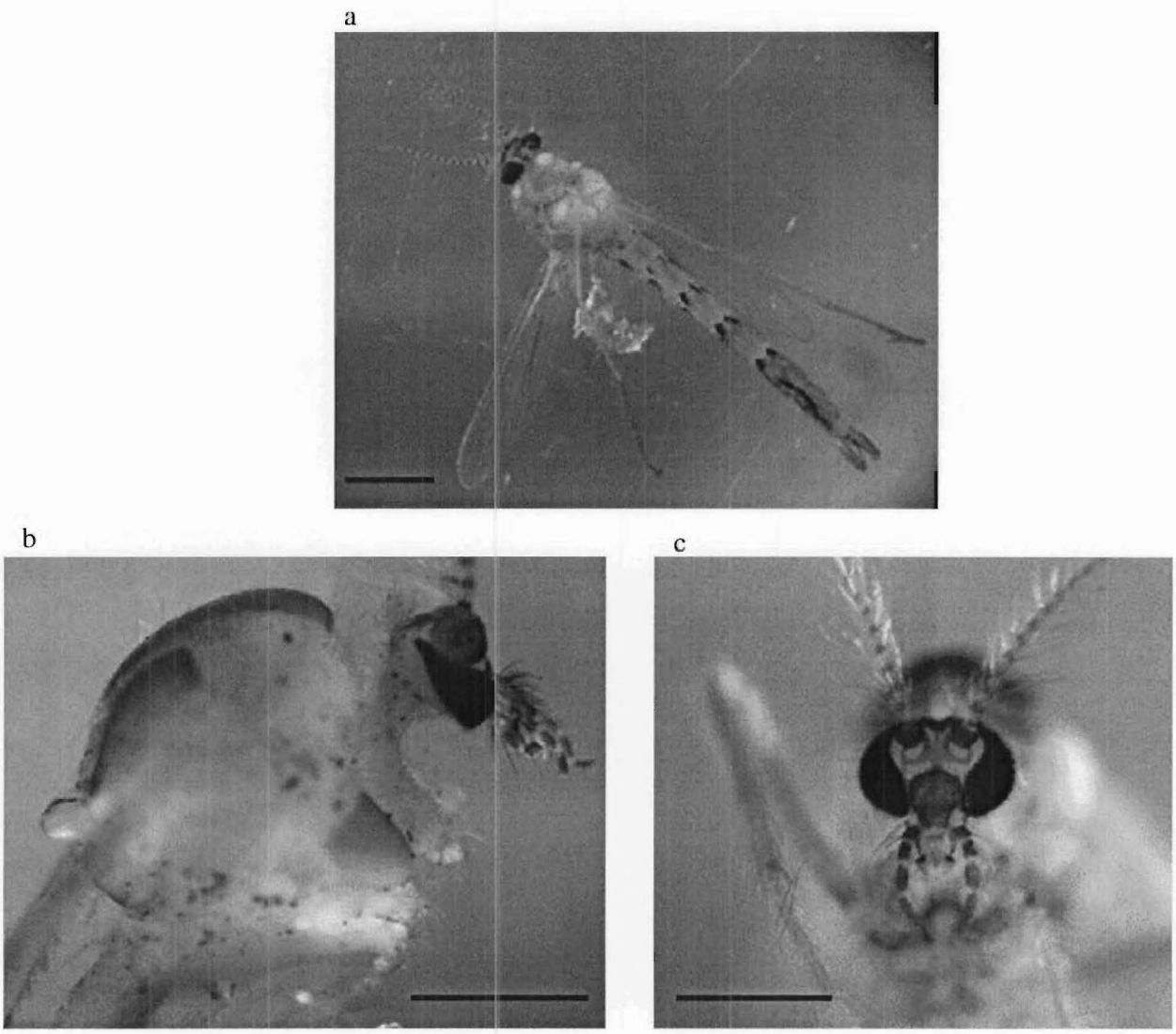

Figure 17. Microscopy images of lake fly (*Chaoborus* sp.). **a)** Ventral view. **b)** Lateral view of thorax and head. **c)** Frontal view of head, showing mouthparts.

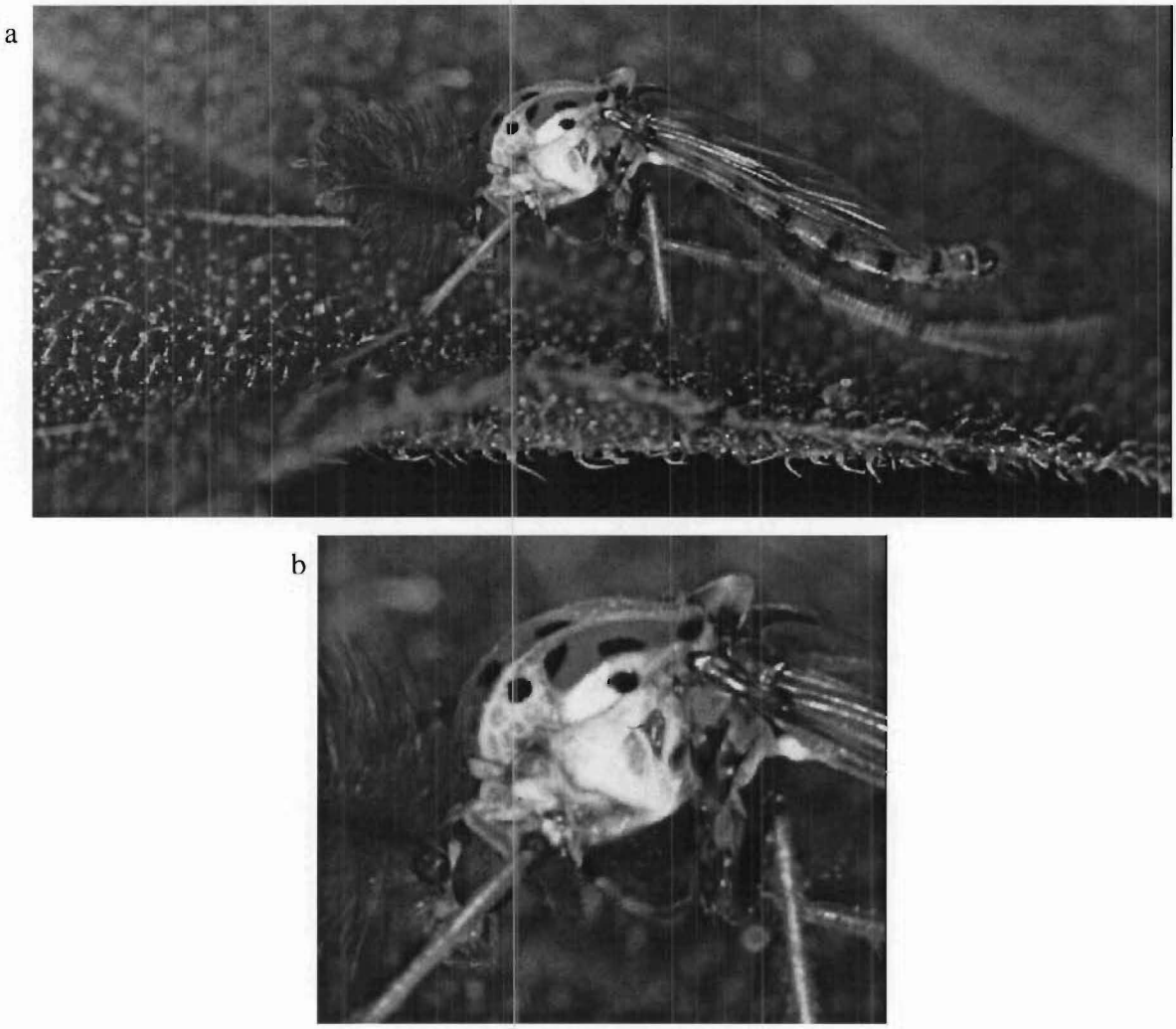

Figure 18. Photographs of *Chaoborus* sp. **a)** Lateral view. **b)** Detail of head and thorax from (a).

Having made the abdomen, the next stage was to create the head of the lake fly. As always, images were used as drawing templates (Fig. 17b, c). The shapes that were to comprise the head (not the mouthparts) were those of the head itself, the two eyes and a scape into which each antenna would eventually be embedded (Fig. 19). Drawing in three-dimensional space is difficult because all objects may appear to be aligned from one perspective, but when viewed from a different perspective the objects may be floating quite randomly through space (Fig. 19-21). Aligning all shapes so that they 'fit' when viewed from every angle can be complicated and having several different views, from different angles, is indispensable for achieving this.

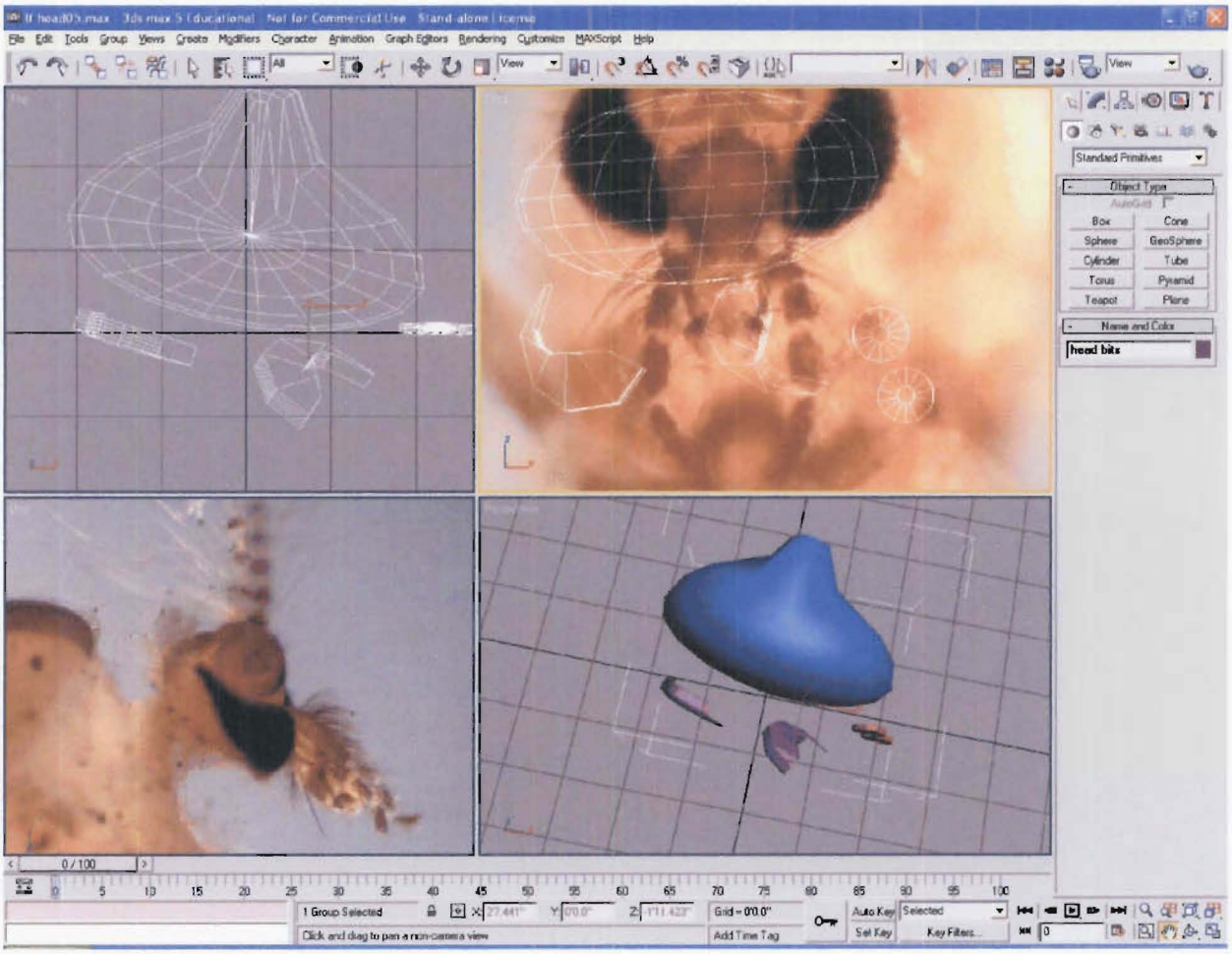

Figure 19. Shapes used to make the head, eyes and antennal scapes of a lake fly.

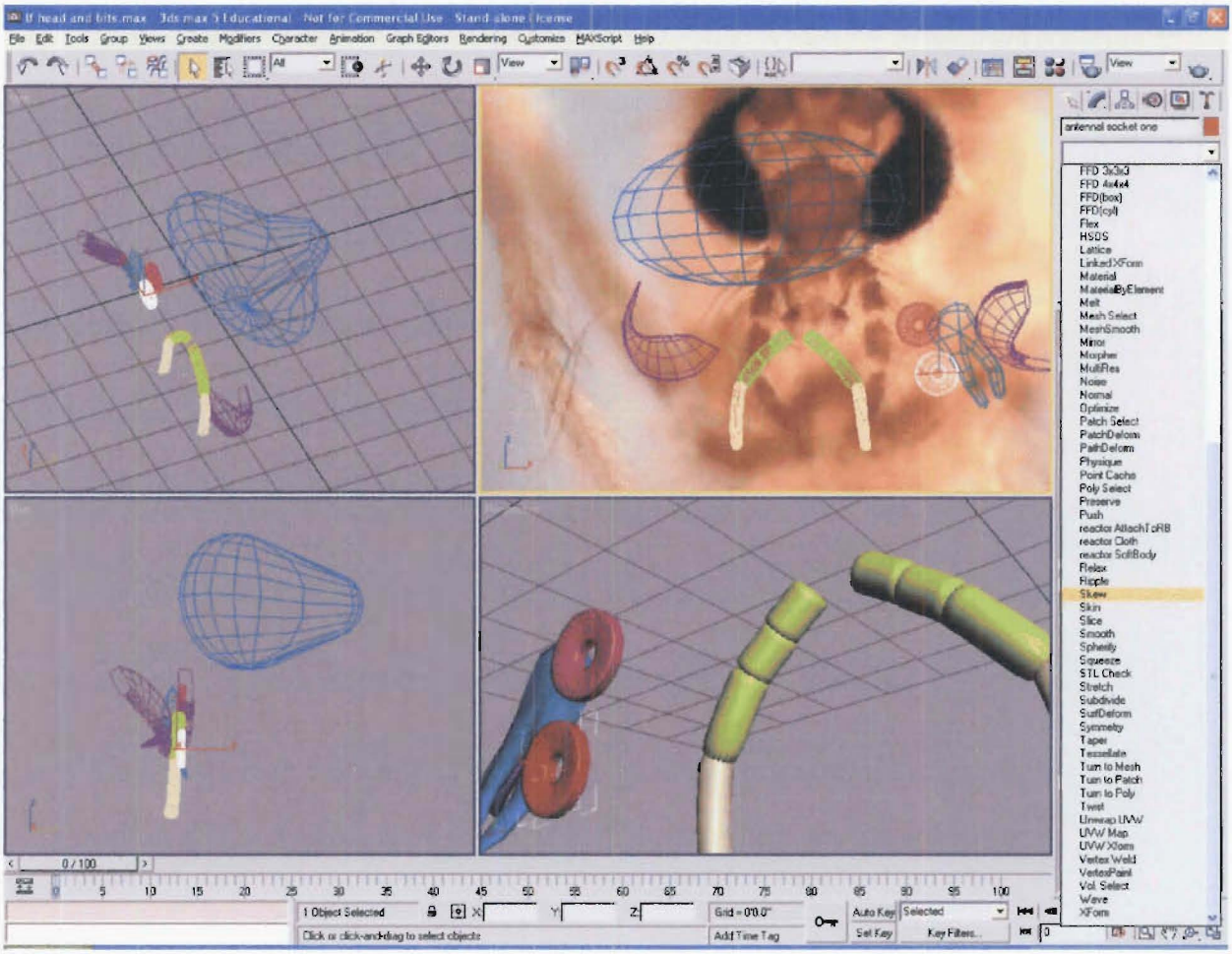

Figure 20. Shapes belonging to head (Fig. 19) and mouthparts of lake fly.

Drawing the mouthparts of the lake fly was the most complex process in the entire procedure of producing the animated lake fly. Figs. 17b and c were used as lateral and frontal views, respectively. However, there were many different parts to draw and many were segmented. In some cases, I used cylinders, which I skewed and smoothed (bottom right window, Fig. 20), and in others I used a box into which I 'cut' at a certain point and subsequently deformed the polygons within the incision, creating a molar-shaped object (blue object visible in the bottom right window, Fig. 20).

Fig. 21 has been inserted to illustrate the objects that comprise a virtual lake fly before they have been aligned into the apparent simplicity of a single 'virtual insect'. At different places in space lie the six green legs, the two dark purple eyes, the green abdomen, the pale mauve thorax, the various mouthparts, the two scapes and the brown head with an abortive dark blue eye embedded in it. Because of the position each object has in space, some of them appear very large, such as the

head, while others, such as the abdomen, appear relatively small in comparison. To fit all these objects together, it is necessary to bring them into a much smaller area, and, if the relative discrepancy of size persists, then objects need to be scaled accordingly.

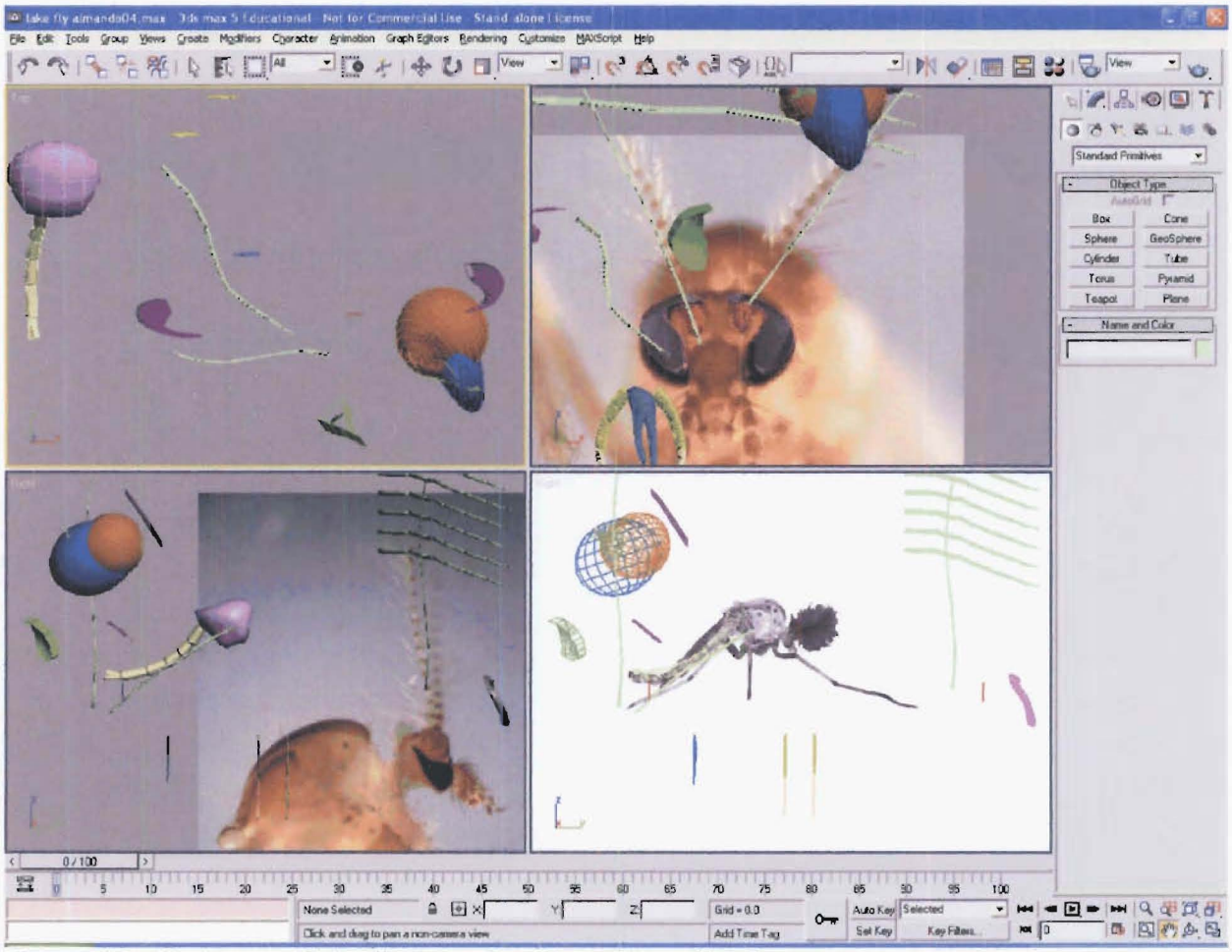

Figure 21. All shapes of lake fly in random three-dimensional space.

After the objects comprising the lake fly have been aligned and scaled, applying the appropriate material to each is necessary. Assigning the right material to each object is important for the appearance of the final, rendered, virtual insect. The apple-shaped thorax of the lake fly, an altered version of the one used for mosquitoes, is shown (Fig. 22) in the process of being assigned a material. A certain set of materials are available with the software, but creating a particular material was sometimes necessary (see Fig. 22a). Once a material has been selected it can be altered in many forms. Materials can be given 'bump', and their opacity, their glossiness, the way they refract light etc., can all be controlled (Fig. 22b).

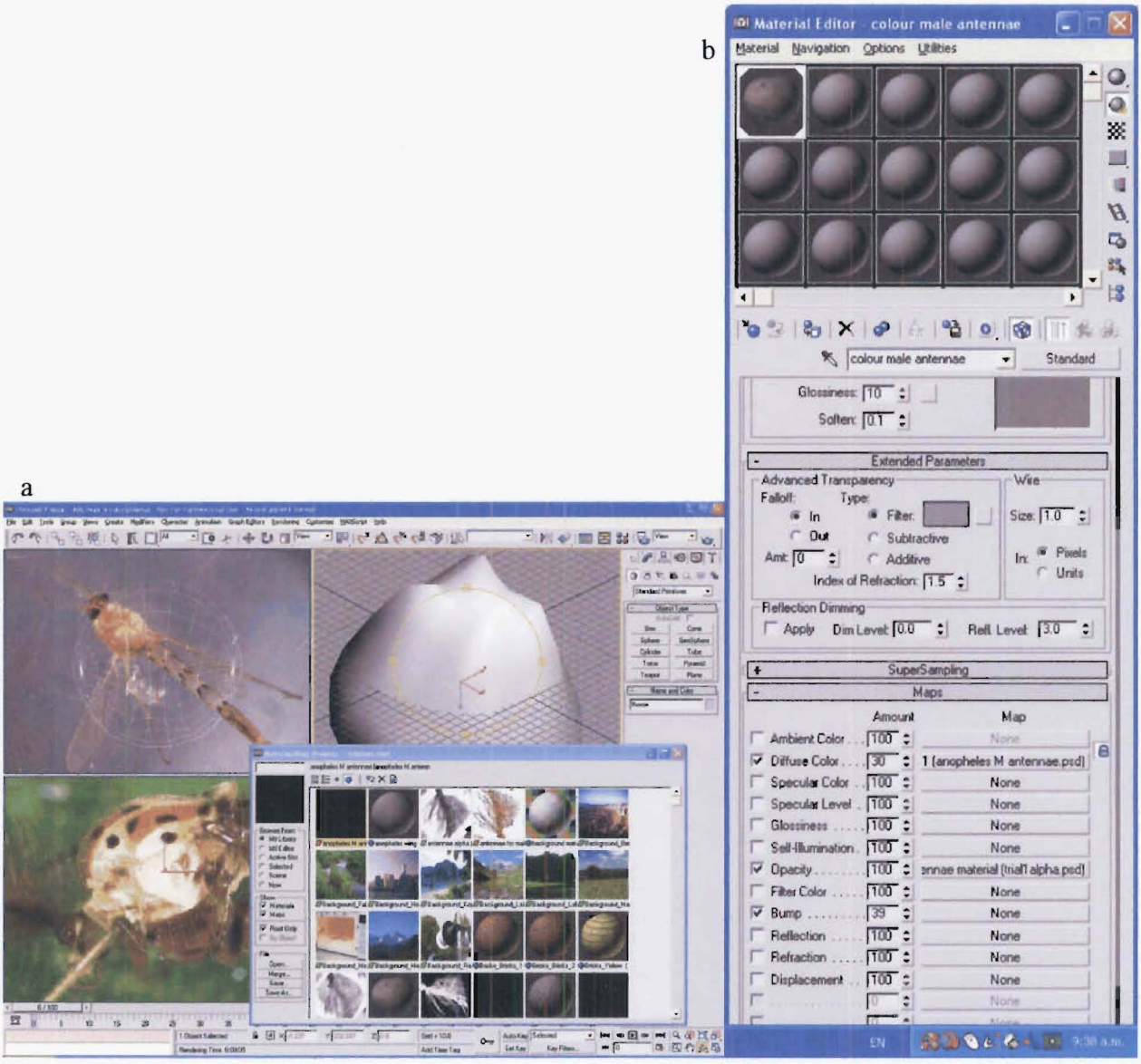

Figure 22. a) Lake fly thorax before having 'material' (inset window) put on it. Note different versions of mosquito antennae visible in top and bottom rows of inset window. **b)** Materials editor, used for altering material properties.

Having assembled the lake fly and assigned materials to it, the process of character-formation and animation were identical to those described for mosquitoes (see Fig. 23 for rendered output of lake flies alongside a blood-fed female mosquito).

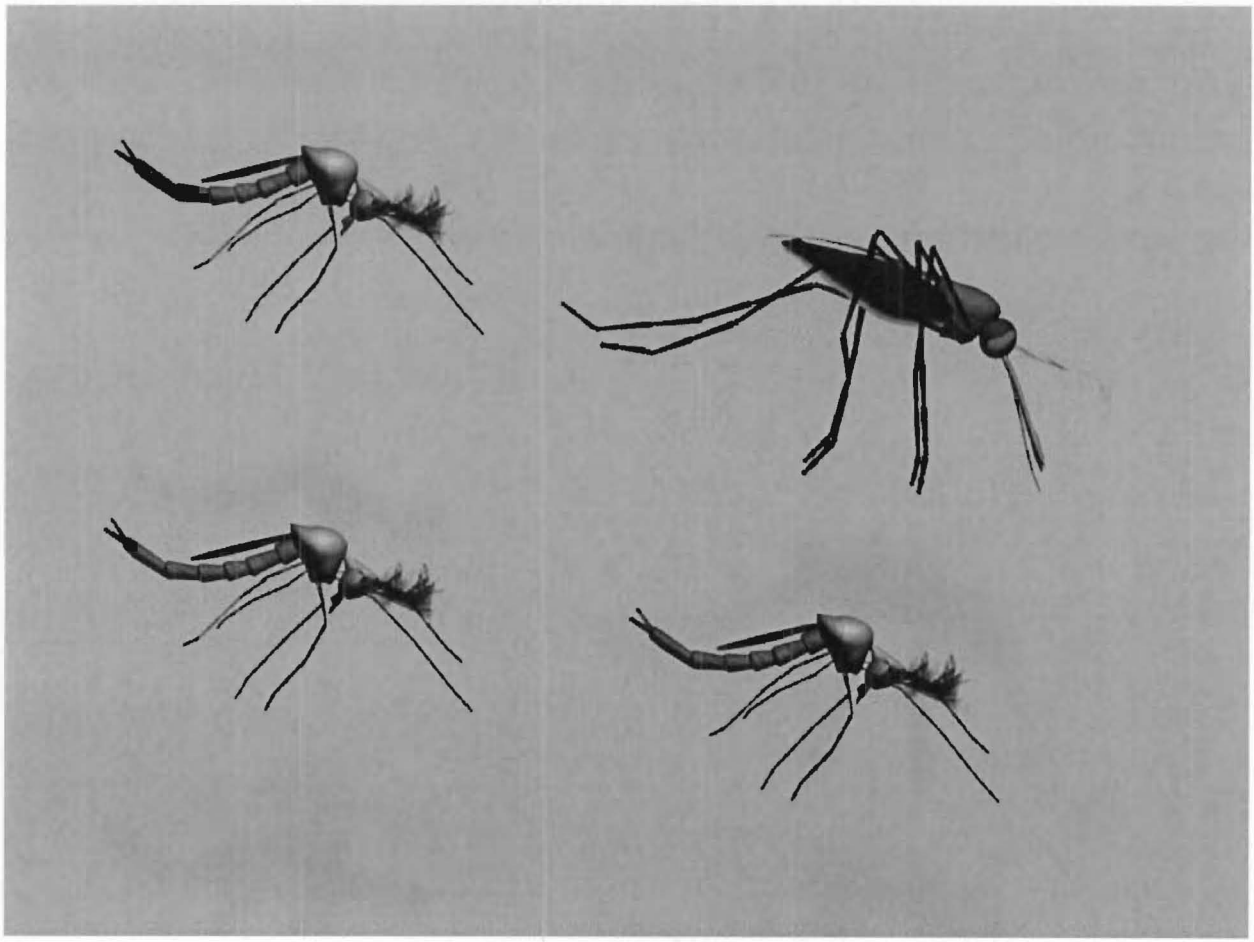

Figure 23. Rendered output of three lake flies and one blood-fed female mosquito.

REFERENCES

Huang, Y. M. 2001. A pictorial key for the identification of the subfamilies of Culicidae, genera of Culicinae, and subgenera of *Aedes* mosquitoes of the Afrotropical Region (Diptera: Culicidae). *Proc. Entomol. Soc. Wash.*, **103,** 1-53.

APPENDIX II
Measurements of the anterior-median eyes of Evarcha culicivora

Materials and Methods

Using an eyepiece micrometer (calibrated using a slide micrometer) on a light microscope, dead spiders and exoskeletons were measured at 4x magnification. Measurements of the diameter of the anterior-median (AM) eye and the width of the carapace at its widest point, between the postero-lateral (PL) eyes, were made to the nearest 25μm. In total, 978 spiders of different ages were measured. Twenty four of these were second instars (first instar after dispersing from the maternal brood sac) but the ages and instars of the other spiders was not known. However, because second instars were identified, it was possible to compare the relative AM eye-size of these small juveniles (n=24) with the AM eye-size of the rest of the spiders (n=954). Results were analysed using t tests and regression (Zar, 1984).

Results

Carapace width was a good predictor of AM diameter (R^2=0.91, P<0.001; Fig. 5). The diameter of the AM eyes of second instars varied between 150 and 200 μm (mean=185.65±3.29 μm; n=24) and the width of the carapace between the PL eyes varied between 500 and 575 μm (mean=544.30±4.57 μm; n=24). The average ratio of AM eye-diameter to carapace width among second instars was 2.95(±0.04). This ratio was slightly bigger than the average ratio of 2.70(±0.01) for the combined data for the rest of the salticids (n=954), indicating that second instar *E. culicivora* have relatively larger eyes than bigger *E. culicivora* (*t*=5.46; P<0.001). When data was pooled across all size classes the ratio of carapace width to AME diameter was 2.71 (±0.01, N=978) (Fig. 1).

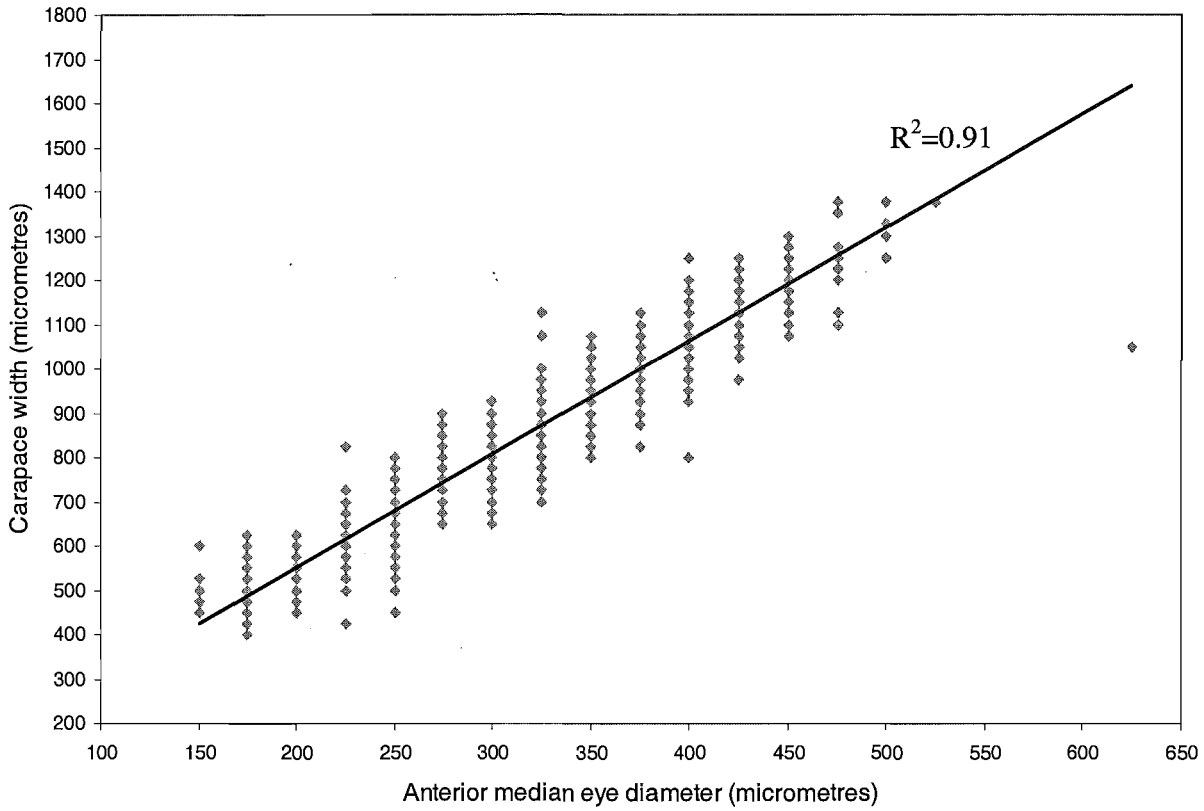

Figure 1. Size of anterior median eyes of *Evarcha culicivora* in relation to carapace width. Eyes measured to nearest 25μm (N=978).